Chapter 1

**General introduction:** The insulin-like growth factor system
Introduction

Salmon and Daughaday first identified insulin-like growth factors (IGFs) as “sulphation factor activity” 36. Later the term “somatomedin” was introduced which was replaced by “insulin-like growth factors” because of their structural homology with insulin 113. The insulin and IGF-family of peptides is important in the normal control of many metabolic and growth related processes 127. The components of the IGF system include IGFs (IGF-I and IGF-II), type I and II receptors, and IGF-binding proteins (IGFBPs).

IGF-ligands

Two different growth factors, IGF-I and IGF-II and variants of each have been identified and isolated from brain extracts and these growth factors are present in the developing rodent and human brain as well as in serum and cerebrospinal fluid (CSF) 62, 63, 123, 124. In the central nervous system (CNS) both growth factors are produced as paracrine or autocrine hormones. Rinderknecht and Humbel 114, 115 revealed the amino acid sequences of both IGF-I and IGF-II in 1978. Human IGF-I and IGF-II, are both single-chain polypeptides with a molecular weight of approximately 7.5-kDa. IGF-I is a basic peptide and contains 70 amino acids, with IGF-II being slightly an acidic protein containing 67 amino acids. The amino acids of both IGF-I and -II are grouped into domains A and B (similar to insulin), domain C (analogous to the connecting peptide of proinsulin) and D (not present in insulin’s). Both IGF factors contain three disulphide bonds, and display approximately 62 percent sequence homology with each other and 47 percent with insulin, which explains the name.

In addition to the classical IGF-I molecules a truncated form of IGF-I known as DES(1-3)IGF-I has been found in foetal and adult human brain 23, 68, 123. DES(1-3)IGF-I is the product of differential processing of pro-IGF-I lacking the first three residues at the amino terminus: Gly-Pro-Glu (GPE). The biologic potency of this truncated form is 10 times higher than that of the full-length form and is explained by reduced binding to IGF-binding proteins 8, 17, 24, 47, 126.
IGF-receptors

The biological effects of IGF-I and IGF-II on target cells are mediated by cell-surface receptors. Two types of IGF-receptors have been identified in human and mammalian brain, the IGF-I receptor and the IGF-II receptor 10, 49, 81 (Figure 1). The insulin receptor (IR) and IGF-I receptor (IGF-IR) are structurally similar; both are members of the type II tyrosine kinase receptor family 27, 131. The IGF-I receptor is a membrane glycoprotein of 300-350-kDa, consisting of two alpha-subunits (135-kDa each) and two beta-subunits (90-kDa each) 82, 108, 137. Disulphide bonds to form a functional heterotetrameric receptor complex connect both alpha and beta-subunits. In analogy with the insulin receptor, IGF-I receptor subunits are encoded within a single 180-kDa-receptor precursor that is glycosylated, dimerised and proteolytically processed to yield the mature alpha-2-beta-2-form of the receptor. The alpha-subunit is entirely extracellular and contains the ligand-binding site, a cysteine-rich domain. The beta-subunit contains the hydrophobic transmembrane domain with a short extracellular region, and a tyrosine kinase domain in its cytoplasmic portion. However, the mature brain also expresses a structural subtype of the IGF-I receptor, which appears to be unique to differentiated neurons. This receptor consists of a lower mass alpha-subunit of 115-kDa 20, 64.

The physiological functions of insulin and IGFs are very different, while their receptor structures are similar. Tissue distribution of the individual receptors, the specificity of the ligand-receptor interaction, the rate of internalisation and the small structural differences between the two receptors explain the well-characterised divergent physiological functions of insulin and IGFs. For example, the liver expresses only the IR and fat cells express mainly IRs. For this reasons these tissues display metabolic responses such as glucose uptake and lipid synthesis. Further, the IGF-IR ligands include IGF-I, IGF-II, and insulin. The IGF-IR binds IGF-I with a high affinity (± 10^{-10}), IGF-II with a 10-fold lower affinity, and insulin with a 100-1000 fold lower affinity 18, 84. On the other hand, IR demonstrates high-affinity binding to insulin (± 10^{-10} M), 10-fold lower affinity binding to IGF-II, and 100-fold lower affinity binding to IGF-I. The IR is mainly involved in short-term metabolic effects, while the IGF-IR is predominantly involved in long-term growth/mitogenic effects 82, 108.

The structural differences between both receptors are also involved in different intracellular signalling. For example, the IR contains two distal tyrosine residues, whereas the IGF-IR contains three tyrosine residues, and the tyrosine kinases of both receptors show distinct preferences for the tyrosine phosphorylation domains of insulin-related substrate (IRS)-1 40.
The IGF-II receptor is structurally and functionally quite different from the insulin and IGF-I receptors (Figure 1). The IGF-II receptor consists of a single glycosylated polypeptide of approximately 260-kDa. Further, the IGF-II receptor is primarily extracellular, having a short cytoplasmic tail and consists of 15 repeat mannose-6-phosphate (M6P)-binding units \(^4, 79, 93, 98\). In 1987, Morgan and co-workers cloned the IGF-II receptor and revealed that it is identical to the cation-independent M6P-receptor \(^98\). This finding was confirmed by showing that the IGF-II receptor binds independently both IGF-II and lysosomal enzymes that contain M6P. Several studies have shown that the binding sites for IGF-II and M6P on the receptor are distinct, and that the receptor can bind both ligands simultaneously \(^90, 103, 118, 129\). The IGF-II receptor binds IGF-II with greater affinity than IGF-I and does not bind to insulin at all. The major functions of IGF-II receptor binding appear to be lysosomal enzyme trafficking and IGF-II degradation via receptor-mediated internalisation \(^79\). There is ample consensus today that mitogenic and biological actions of both IGF-I and IGF-II are mediated by the IGF-I receptor.
Introduction

Signalling via IGF-I receptors

Binding of IGF-I and or IGF-II to the IGF-I receptor induces receptor autophosphorylation of their beta-subunits and results in activation of the intrinsic tyrosine kinase activity of the IGF-I receptor and the subsequent tyrosine phosphorylation of several substrates including IRS and Shc. The IRS are known as “docking” proteins and constitutes a family of four structurally related adapter proteins that can link the IGF-I receptor to downstream signal transduction mediators regulating cellular growth. IRS-1 is the most extensively studied, and has multiple tyrosines, which associates with SH2 domain-containing proteins including growth factor receptor bound-2 protein (grb2) and p85-subunit of phosphoinositol-3 (PI-3) kinase.

Signalling via the mitogen-activated protein (MAP) kinase cascade

Stimulation of grb2 by activated IRS-1 leads to the coupling of grb2 with the guanyl nucleotide exchange factor sos, which in turn activates ras. In the ras pathway, phosphorylation of c-raf 1 activates the downstream protein kinase, MAP kinase kinase 1 (also known as MEK or M KK1) or MAP kinase kinase 2 (M KK2). M KK1 and M KK2 activate two members of the MAP kinase family; extracellular signal related protein kinase (ERK)-1 and -2. Activated ERK-1 and (or) ERK-2 are translocated to the nucleus where they phosphorylate a variety of transcription factors including c-fos, c-jun and c-myc.

This signalling cascade is important for cellular growth and mitogenesis. IGF-I receptor signalling via the MAP kinase cascade induces neurite outgrowth, changes in growth cone associated protein-43 (GAP-43) and c-myc gene expression. The c-myc protooncogene encodes a nuclear transcription factor that is both necessary and sufficient to trigger entry of certain cell types into the S-phase of the cell cycle. Inhibition of ERK activation with the MEK inhibitor PD98059 blocks the effects of IGF-I mediated IGF-I receptor activation, and indicates that MAP kinase activation induces neuronal differentiation.

Signalling via the serine-threonine protein kinase Akt pathway

IGF-I activated IRS-1 binds the p85 alpha-subunit PI-3 kinase, and this complex activates the 110-kDa catalytic subunit, which phosphorylates phosphoinositides, generating PI-3 phosphate, PI-3, 4-bisphosphate, and PI-3, 4, 5-triphosphate. These phosphatidyl-inositides in turn activate protein kinases including p70 ribosomal protein S6 kinase (p7066k)
and Akt as well as other less characterised downstream molecules. This pathway is important for a) IGF-mediated survival b) enhancement of cellular motility and c) protection from programmed cell death.

**Figure 2**

Schematic presentation of the intracellular-signalling pathways of the IGF-I receptor. The initial response of the IGF-I receptor to IGF-I binding is to undergo autophosphorylation on specific tyrosine residues. The intrinsic tyrosine kinase activity of the IGF-I receptor is enhanced and phosphorylates multiple substrates, including IRS1 and IRS2 on tyrosine residues. Proteins containing SH2-domains such as PI-3 kinase, grb2, and syp associate with specific phosphotyrosine-containing motifs within IRS1 and IRS2. A similar response occurs with shc. The ras/raf/MAP kinase pathway, for example is activated by the association of IRS1 and shc with grb2, which in turn is associated with the mammalian guanine nucleotide exchange factor protein sos.
Dudeck and co-workers found that insulin and IGF-I efficiently activate PI-3 kinase in neurons. Effects of PI-3 kinase on cell survival are mediated by the serine-threonine kinase Akt (protein kinase $\beta-\alpha$). Akt contains at its amino-terminus a domain termed the pleckstrin homology (PH) domain, which may regulate the activation of Akt by binding D3-phosphorylated phosphoinositides that are the products of PI-3 kinase. Activation of Akt was blocked by the PI-3 kinase-inhibitor wortmannin LY2944002, which suggested that this activation was dependent on PI-3 kinase 44.

Insulin-like growth factor binding proteins

In the circulation

IGFs (IGF-I and IGF-II) are unlike traditional endocrine hormones, such as growth hormone (GH) and insulin, which are secreted from storage vesicles in an endocrine gland in response to releasing factors such as GH releasing factor or glucose. In contrast, the IGFs are not stored in an endocrine gland and are therefore not released acutely into the blood by releasing factors. However, the IGFs are stored in the blood and in tissues in a different, and somewhat unique manner for a protein hormone; they are stored bound to specific IGFBPs. The IGFBPs comprise a family of six proteins with the common property of binding IGF-I and IGF-II 109. The diverse activities of IGF-I and IGF-II are mediated by IGF-I receptors and modulated by IGFBPs.

IGFs synthesised in the liver are secreted into the general circulation as endocrine factors. The proposed functions of IGFBPs which regulates the endocrine actions of IGFs are: 1) inhibition of insulin-like activity; 2) prolongation of the plasma half-lives of IGFs and 3) regulation of the rate of transport of IGFs from the vascular compartment to enhance the growth-potentiating effects of IGFs 58, 74, 144. The major pool of IGFs circulates in human serum as a 150-200-kDa complex. These large complexes consist of a) IGF-I or IGF-II, b) 38-42-kDa acid stable protein (IGFBP-3), and c) an 86-kDa acid-labile subunit (ALS-protein) 11, 12. The other 20-25 % of the IGFs is associated with lower mass IGFBPs. Less than 1 % of IGFs are found in the free form 14, 59, 61. The major form of binding protein present in the circulation is IGFBP-3. IGFBP-3 is glycosylated and binds IGF-I and IGF-II with similar affinities. IGFs are stabilised when they are complexed with IGFBP-3 and the ALS-protein 12, 107. The ALS-protein plays an important role in regulating the affinity of IGFBP-3 to IGF-I,
thus regulating the levels of free IGFs. In addition, ALS-proteins increase the molecular mass of the IGF-IGFBP-3 complex so that the access of the circulating IGFs to the extracellular fluid and thus to the various tissues is limited. IGFs are released from the 150-kDa complexes by limited proteolysis of IGFBP-3 and cross the vascular endothelial barrier alone or associated with smaller IGFBPs \(^{13,107}\). Other binding proteins in human serum and biological fluids are IGFBPs-1, -2, -4, -5 and –6. IGFBP-2 is the major binding protein in CSF and is a non-glycosylated protein of 31-36-kDa \(^{116}\). Characteristics of these IGFBPs are summarised in Table 1.

<table>
<thead>
<tr>
<th>IGFBP</th>
<th>Molecular mass (kDa)</th>
<th>IGF affinity</th>
<th>modulation of IGF-action</th>
<th>Source in biological fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1</td>
<td>30</td>
<td>I = II</td>
<td>inhibition and/or potentiation</td>
<td>amniotic fluid, serum, placenta, endometrium, milk, urine, synovial fluid, interstitial fluid, and seminal fluid.</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>31-36</td>
<td>II&gt;I</td>
<td>inhibition</td>
<td>CSF, serum, milk, urine, synovial fluid, interstitial fluid, lymph, follicular fluid, seminal fluid, and amniotic fluid.</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>38-43</td>
<td>I = II</td>
<td>inhibition and/or potentiation</td>
<td>serum, follicular fluid, milk, urine, CSF, amniotic fluid, synovial fluid, interstitial fluid, and seminal fluid.</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>32-36</td>
<td>I = II</td>
<td>inhibition</td>
<td>serum, follicular fluid, seminal fluid, interstitial fluid, and synovial fluid.</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>29</td>
<td>II &gt; I</td>
<td>potentiation</td>
<td>serum and CSF</td>
</tr>
<tr>
<td>IGFBP-6</td>
<td>34</td>
<td>II &gt; I</td>
<td>inhibition</td>
<td>CSF, serum and amniotic fluid.</td>
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</tbody>
</table>

(Taken from Rajaram et al., 1997).
Modulation of IGFBPs

Previous studies have identified the expression of all six IGFBPs in the brain, but the cell types expressing these IGFBPs remain unclear. In many cases the expression pattern of IGFBPs is spatially and temporally co-ordinated with IGFs. IGFBP-2 mRNA is localised in astrocytes surrounding neurons which express IGF-I, and IGFBP-5 mRNA is found in neurons of the sensory relay system together with IGF-I mRNA. These observations suggest that locally synthesised IGFBPs modulate the effects of IGFs in an autocrine or paracrine manner. IGFBPs have been shown to have both inhibitory and stimulatory effects on the actions of IGFs and may regulate the role of IGFs in growth, differentiation and survival in the CNS (Figure 3).

All six IGFBPs inhibit IGF action by sequestering IGFs, thereby preventing the interaction of IGFs to IGF-receptors and by inhibition of IGF-induced DNA synthesis in various cell types. Compared to IGF-I receptors, IGFBPs have 10 to 100 fold higher affinities for IGFs, and bind IGFs to decrease local free IGF availability. IGFBP-2 inhibits the ability of IGFs to stimulate DNA synthesis in various cells, and IGFBP-2 prevents binding of IGFs to the cell surface and limits the accessibility of IGFs to receptors. Growth arrest of human neuroblastoma cells and human keratinocytes is caused by increased expression of IGFBP-6 mRNA and protein. The growth inhibitory action of IGFBP-4 on bone cells is involved in the pathogenesis of osteoporosis. Circulating levels of IGFBP-4 are high in older-age subjects and correlate with parathyroid hormone levels. Parathyroid hormone regulates IGFBP-4 in bone cells by increasing IGFBP-4 synthesis and inhibiting IGFBP-4 proteolysis. Estradiol reverses these actions by enhancing IGFBP-4 proteolysis and therefore increasing IGF-I bioavailability.

The phosphorylation-state of IGFBPs influences the affinity to IGF-I, therefore modulating IGF-I actions. Serine-phosphorylation of IGFBP-1 and IGFBP-3 increases its binding affinity for IGF-I seven-fold and favours inhibition whereas non-phosphorylated IGFBP-1 and IGFBP-3 has the opposite effect and potentiates the effect of IGF-I on DNA synthesis in vitro.

Other mechanisms involved in IGF regulation are specific IGFBP-proteases. IGFBP-degrading proteases secreted by cells have been proposed to act as growth stimulators by increasing local IGF availability. Circulating and tissue specific IGFBPs bind IGFs, thereby forming biologically inactive complexes. IGFs can be released from these complexes by proteolysis. Potentiation of IGF activity may occur under specific circumstances, and involves the slow dissociation of IGFs from IGFBPs. IGFBP fragments generated by the action of
cellular endoproteases show a marked loss of IGF binding. Members of several classes of proteases have shown the ability to cleave IGFBPs, including kallikreins, cathepsins and matrix metalloproteinases. For example, prostate-specific antigen (PSA) is a kallikrien-like serine protease present in seminal plasma that causes proteolysis of IGFBP-3 and IGFBP-5, and PSA decreases IGFBP-3 induced cell growth inhibition. Proteolysis of IGFBP-4 is regulated by IGF-II, and IGF-II induced IGFBP-4 proteolysis increases the local availability of IGF-I that leads to stimulation of cell growth.

Another way to modulate IGF actions is the binding of IGFBPs to cell surfaces. Binding of IGFBPs to the extracellular matrix (ECM) or cell membranes facilitates the release of IGF leading to enhanced delivery of IGF to receptors and increased IGF mitogenic actions. IGFBP-3 binds to specific cell receptors and favours the slow release of IGF-I, avoiding IGF-I receptor down regulation.

**Figure 3**

Demonstrated actions of IGF-dependent and IGF-independent IGFBPs. IGFBPs inhibit IGFs by forming inactive complexes. IGFs are freed from these complexes when proteases degrade IGFBPs or when IGFBPs interact with cell surfaces or ECM. Binding of IGFBPs to integrins and or unknown protein receptors modulates IGF-independent actions.
IGFBP-5 potentiates IGF-I-stimulated DNA and protein synthesis in various tissues. Potentiation requires the binding of IGFBP-5 to the ECM, which protects it from proteolytic cleavage and reduces the affinity to IGFs 10-fold, approximately equal to that of the IGF-I receptor. Furthermore, the formation of IGF-I/IGFBP-5 complexes is inhibited by heparin that alters the binding affinity of IGFBP-5 for IGF-I. Heparin in turn modulates IGFBP-5 membrane binding. Russo and co-workers showed that IGFBP-2 is associated to cell surface proteoglycans in the olfactory bulb of the rat brain. The binding of IGFBP-2 to chondroitin-6-sulphate decreases the binding affinity of IGFBP-2 to IGF-I 3-fold. They suggest that cell associated IGFBP-2 may have a role in directing IGFs to specific sites in the brain. Further, it is also been demonstrated that IGFBP-1 stimulates cell migration and cell promotion in an IGF-independent way through interaction with the alpha-5-beta-1-integrin. In contrast, IGFBP interaction with cell surface structures is not always associated with IGF-induced receptor activation. The binding of IGFBP-3 to cell-associated proteins has been described in a variety of cells and is correlated with the ability of IGFBP-3 to cause growth inhibition. IGF-I is able to block the association of IGFBP-3 to cell membranes, and reverses IGFBP-3-induced growth inhibition in breast cancer. Only IGFBP-3 associates to cell membranes and IGF-I/IGFBP-3-complexes cannot, suggesting that IGF-I is able to overcome the growth inhibitory effects of IGFBP-3 by binding IGFBP-3 and not to its receptor.

In conclusion, IGFBPs can exert IGF-dependent and IGF-independent actions in different ways. The multitude of effects depends on the cell type in which they are expressed, how they interact with cell surfaces and ECM, the presence of specific proteases and the formation of high affinity binding proteins complexed with IGFs.

IGF-I in the central nervous system

IGF-I is a neurotrophic growth factor with insulin-like metabolic activities, and possesses potential clinical applications, particularly in neurodegenerative disorders and multiple sclerosis (MS). IGF-I is widely distributed in the developing and mature CNS, and these growth factors play an important role in neuromodulation, survival and differentiation of glial cells and neurons, glial cell growth and neuronal outgrowth (Table 2).
There are developmental and tissue-specific expression patterns of IGF-I in the CNS and peripheral nervous system (PNS) \(^7\). During early foetal development IGF-I expression is high and declines in the adult CNS \(^16\). However IGF-I is still present in the adult CNS and in neurons and Schwann cells of the PNS \(^60,119\). In the adult CNS, neurons and glia synthesise IGF-I mRNA, in contrast only glia produce IGF-II mRNA \(^3,89,119,122\). In adult rat and human brain, IGF-I mRNA is found in different regions such as hippocampus, olfactory bulb, striatum, brainstem and cerebellum \(^119\). Astrocytes express both IGF-I and IGF-II \(^3,122\). Premature oligodendrocytes express IGF-I only and this expression is down regulated in mature oligodendrocytes \(^125\). Especially astrocytes surrounding lesioned parenchyma contain high levels of IGF-I and this observation suggests that brain injury mechanisms might induce a specific pattern of IGF-I expression in a subpopulation of astrocytes \(^50\).

MS is an inflammatory demyelinating disease of the CNS \(^104\). The pathological hallmarks of MS are the degeneration and loss of oligodendrocytes, as well as the destruction of myelin sheets. Amyotrophic lateral sclerosis (ALS) is a progressive, devastating disease of the CNS that affects both upper and lower motor neurons \(^120\). The pathological hallmarks of ALS are the degeneration and loss of motor neurons accompanied by astrocytic gliosis and intraneuronal inclusions \(^28,101\). In both diseases, the underlying mechanism that causes the death of oligodendrocytes and motor neurons remains unknown. Promotion of remyelination in MS and the rescue of motor neurons in ALS are potential strategies for therapeutic intervention.

The use of IGF-I in MS has been proposed due to its key role in the maturation and survival of oligodendrocytes, which are essential for the synthesis of myelin. Several studies demonstrated that IGF-I induces oligodendrocyte development and myelination \(^96,97\), and doubles the number of myelinating oligodendrocytes in the rat anterior medullary velum (AVM) by promoting survival and differentiation of early oligodendrocytes \(^9,21,53\). During primary myelination, IGF-I inhibits apoptosis of mature oligodendrocytes \(^92\), and oligodendrocyte-derived IGF-I supports neuronal survival \(^134\). Transgenic mice that overexpress IGF-I have shown increased brain growth and myelination \(^25,140,141\). Carson et al., (1993) demonstrates that the brains of transgenic mice were 55 % larger and the myelin content was 130 % greater compared with controls \(^25\). Oligodendrocyte number as a percentage of total cell number was not increased in the transgenic mouse brains, indicating that an increase of myelin was primarily the result of an increase of myelin production per oligodendrocyte. IGF-I has already been used in animal models for the study of MS. Such an animal model that mimics an inflammatory condition is experimental autoimmune
encephalomyelitis (EAE)\textsuperscript{86}. Administration of IGF-I in EAE significantly reduces lesion severity and demyelination accompanied by clinical improvement\textsuperscript{138, 139}. Prolonged administration of IGF-I has indicated a role for IGF-I as an anti-inflammatory factor, which reduces disease severity. The anti-inflammatory effects of IGF-I were not dependent on components of the inflammatory process that are associated to myelin breakdown. These effects were mediated by IGF-I to reduce the blood-brain barrier (BBB) defects and the permeability of the BBB to immune effector cells, cytokines and serum proteins\textsuperscript{88}.

IGF-I is not only a growth promoting and survival factor for oligodendrocytes but also plays a vital role in the CNS for the development, differentiation and survival of neurons. In the adult nervous system, IGF-I is a neuromodulator and regulates synaptic plasticity\textsuperscript{130}. IGF-I has been proposed as a therapy for the treatment of motor neuron diseases (MND), including ALS\textsuperscript{85}. IGF-I protects motor neurons from axotomy-induced death\textsuperscript{41, 70}, in contrast to other neurotrophins and cytokines that are markedly less effective\textsuperscript{87}. \textit{In vitro} and \textit{in vivo} experiments have shown that IGF-I increases long-term neurite outgrowth, branching and synapse formation\textsuperscript{46}, and both IGF-I and -II stimulates motor neuron survival and nerve sprouting in targeted muscles\textsuperscript{22, 38, 51, 69, 102, 143}. IGF-I is also involved in the response of neuronal injury and protects neurons against various neurodegenerative stimuli and neurotoxic insults. IGF-I protects a) cerebellar granule cells and hippocampal neurons against kainic acid and iron toxicity b) hippocampal neurons from beta-amyloid c) striatal neurons from quinolinic acid and d) dopaminergic neurons against 6-hydroxydopamine\textsuperscript{1, 5, 42, 57, 83, 145}.

It has been reported that administration of IGF-I into CSF and intracerebroventricular reduces neuronal death following hypoxic-ischemia (HI) brain injury, and HI-brain injury accelerates the penetration of IGF-I through different parts of the CNS\textsuperscript{54, 55, 56}. This raises the question whether IGFs that are present in the circulation can pass through the BBB and enter the brain. Several studies have demonstrated that IGFBPs and IGF-IR are present in brain parenchyma and that these proteins could act as specific transporters of the IGFs across the BBB, although this concept is still unclear and requires further investigation\textsuperscript{45, 112}.

In conclusion, these observations suggest that IGF-I has a wide spectrum of actions in the CNS and is may be a good candidate for the treatment of ALS and MS.
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<tr>
<th>Organ</th>
<th>Action of IGF-I</th>
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<tr>
<td><strong>Brain</strong></td>
<td>Promotes division, differentiation, maturation and assures survival or reduces apoptosis of neuronal (olfactory bulb, cortex, hypothalamus, hippocampus, mesencephalic, brainstem, cerebellum) and glial (Schwann, oligodendrocytes, astrocytes) cells.</td>
</tr>
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<td>Protects neurons against toxicity induced by: iron, colchine, Ca^{2+} destabilises, ( \text{H}_2\text{O}_2 ), amyloid peptides, human amylin and cytokines.</td>
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<td>Modulates release of neurotransmitters: acetylcholine, dopamine, serotonin, glutamate, neuropeptide Y.</td>
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<td></td>
<td>Induces the expression of neurofilament, tubulin, and myelin basic protein.</td>
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<td>Directs the sprouting of spared afferents into a deafferented hippocampus.</td>
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<td></td>
<td>Actions on glucose metabolism.</td>
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<tr>
<td><strong>Spinal cord</strong></td>
<td>Modulates choline acetyltransferase activity and attenuates loss of cholinergic phenotype.</td>
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<td></td>
<td>Enhance motor neuron sprouting.</td>
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<td>Increases myelination and inhibits demyelination.</td>
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<td>Stimulates interstitial proliferation.</td>
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<td></td>
<td>Reduces apoptosis in motor neurons during normal development, spinal transection, and deafferentiation.</td>
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<tr>
<td></td>
<td>Potential chemotactic properties.</td>
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<td></td>
<td>Stimulates motor neuron proliferation and differentiation from precursor cells.</td>
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<td></td>
<td>Promotes survival of Schwann cell precursors.</td>
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<tr>
<td></td>
<td>Promotes Schwann cell division, maturation and growth.</td>
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<tr>
<td></td>
<td>Increases the rate of regeneration of sciatic sensory and motor nerves.</td>
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<td></td>
<td>Faster recovery after crush or freeze injury of the nerve.</td>
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<td></td>
<td>Prevents peripheral neuropathies induced by cancer chemotherapy.</td>
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Aim and outlines of this thesis

The aim of this thesis is the investigation of the IGF system in the CNS with a primary focus on MS and ALS. The IGF system comprises more than IGF-I and IGF-II alone. The system consists of two different IGF-receptors, which mediate IGF-actions and at least six IGFBPs that modulates IGF-actions. Clinical application of IGF-I as a treatment for ALS and MS requires a good understanding of the IGF-system. Therefore, we characterised the presence of the different IGF-components in the circulation and in the normal and affected CNS.

In chapter 2 the distribution of IGF-I receptors in the normal human brain and pituitary gland were studied by autoradiography. In this study it became apparent that several well-defined brain structures are enriched with IGF-I receptors suggesting a neurotrophic/survival role of IGFs on specific neuronal systems, and IGF-I receptors observed in the white matter may be associated with oligodendrocytes.

In chapter 3 the characteristics of the IGF-I receptor in human brain and the anterior pituitary gland were studied by using two different IGF-I analogues. DES(1-3)IGF-I and R3-IGF-I show low affinity binding to IGF-binding proteins. In this study, we revealed that IGF-I in the anterior pituitary was not bound to IGF-I receptors but to a cell membrane associated IGFBP-3-acid-labile subunit.

In chapters 4 and 5 the distribution of IGF-I and –II receptors were studied by using iodine-labelled IGF-I and -II in the MS affected brain. IGF-I receptors were present in the white matter of MS patients and in MS lesions, suggesting that these receptors play an important role in myelination and astrogliosis. In contrast, IGF-II receptors were not found in MS lesions or in the white matter of controls. These results suggested that IGF-II receptors in human brain were not involved in astrogliosis.

In chapter 6 the major components of the circulating IGF-system were investigated in MS patients and controls by using radioimmunoassay (RIA) and enzyme immunoassay (EIA). In this study it became apparent that the different components of the IGF-system in serum and in CSF were not different between MS patients and controls.
In chapters 7 and 8 the presence of IGFBPs on different glial cells was studied in the normal white matter and in MS lesions by using double-labelling experiments. Binding proteins were upregulated in MS lesions and these results indicated that high levels of IGFBPs inhibit or stimulate IGF-I actions. \textit{In vitro} experiments revealed that IGFBPs upregulated in oligodendrocytes of MS patients inhibited IGF-I induced myelin formation and reduced IGF-I mediated cell survival. These results indicated that enhanced expression of IGFBPs reduces the bioavailability of IGF-I, and may play a role in the loss of oligodendrocytes and failure of remyelination.

In chapter 9 we studied the different components of the IGF-I system in spinal cord sections of ALS patients. Free IGF-I was drastically reduced in ALS patients, and there was enhanced immunoreactivity for IGFBPs and IGF-I receptors. These results indicated that IGF-I binding to IGFBPs reduces the bioavailability of IGF-I in ALS patients and this abnormality may play an important role in the processes that lead to motor neuron death.

In chapter 10 we summarised the results of this thesis, and discussed each chapter followed by concluding remarks.
References


Chapter 1


