Molecular Neurobiology
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ISSN0893-7648/03/28(1): 23–49/$25.00

Cell Death in the Nervous System

Lessons from Insulin and Insulin-Like Growth Factors

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Abstract

Programmed cell death is an essential process for proper neural development. Cell death, with its similar regulatory and executory mechanisms, also contributes to the origin or progression of many or even all neurodegenerative diseases. An understanding of the mechanisms that regulate cell death during neural development may provide new targets and tools to prevent neurodegeneration. Many studies that have focused mainly on insulin-like growth factor-I (IGF-I), have shown that insulin-related growth factors are widely expressed in the developing and adult nervous system, and positively modulate a number of processes during neural development, as well as in adult neuronal and glial physiology. These factors also show neuroprotective effects following neural damage. Although some specific actions have been demonstrated to be anti-apoptotic, we propose that a broad neuroprotective role is the foundation for many of the observed functions of the insulin-related growth factors, whose therapeutical potential for nervous system disorders may be greater than currently accepted.

Index Entries: Insulin; proinsulin; insulin-like growth factors; cell death; apoptosis; nervous system; neural processes; neurodegeneration; neuroprotection; neurorepair.

Physiological and Pathological Cell Death in the Nervous System

Programmed cell death is essential to neural development. Neuron populations are thus selected by cell death to adjust numbers to the

Received 12/18/02; Accepted 2/28/03
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target fields and to refine connections *(1–4).* Programmed cell death also affects neural precursors and immature neurons, and its impact in the early stages of neural development has not yet been determined *(4,5).* Developmental cell death in the nervous system appears to rely—although not exclusively—on the availability of extrinsic survival promoters, including various families of growth factors and neurotransmitters, and on electrical activity *(5–9).* More recently, extrinsic death inducers have been characterized *(10–14).* Extrinsic signals control the intracellular balance of pro-survival and pro-death regulators (among these, the best-characterized are Bcl-2 family members, ceramide/ceramide-1-phosphate, Apaf-1, mitochondrial death inducers, and IAPs), eventually resulting in the activation of cell-death executors (caspases, other proteases, and endonucleases, among others). For a detailed description of the cell-death program, *see* refs. *1,15–17.*

Cell death appears to be critical for the origin or progression of multiple pathologies, including neurological diseases caused by traumatic injury, genetic disorders, and age-related degeneration. Neurons and glial cells are abnormally lost in neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, and Parkinson's disease, frontotemporal dementia, amyotrophic lateral sclerosis, stroke, and many other pathologies, thus impairing normal neural function. It is currently believed that understanding the mechanisms of cell-death regulation during neural development may contribute to an understanding of cell death under pathological conditions, thus providing targets and tools to induce neuroprotection and neurorepair *(18–23).*

Insulin is a classical component of defined culture media *(24).* Cultures of primary neurons and neural stem cells are typically maintained at high, micromolar insulin concentrations *(25,26).* This high insulin concentration is believed to act by binding to the insulin-like growth factor-I (IGF-I) receptor. We believe that this useful and widespread tissue-culture "trick" has delayed an indepth analysis of the genuine roles of these factors in neural-cell physiology. Furthermore, the molecular and cellular basis of insulin-induced neuroprotection in vitro are poorly defined. In the first part of this article, we summarize the growing number of studies showing that the insulin-related growth factors (proinsulin, insulin, IGF-I, and IGF-II), especially IGF-I, are essential for the development, maturation, and normal function of the vertebrate nervous system. Earlier reviews partially covered this goal *(27–30).* In the second part of this article, we summarize the effects of insulin-related growth factors on neural physiology, mainly during development, to extract lessons and propose theories on the possible role of these factors in neuroprotection.

The Insulin-Related Growth-Factor System

The peptidic factors insulin (and its precursor proinsulin), IGF-I, and IGF-II, their cellular receptors, and the specific IGF-binding proteins form the insulin-related growth-factor system (Fig. 1), which is involved in modulating many physiological processes, including development, growth, reproduction, aging, and metabolism. Other distantly related factors have recently been described in vertebrates and invertebrates *(34–43).*

Insulin was the first protein sequenced in the early 1950s. IGF-I, initially known as somatomedin-C, was sequenced in the 1970s and renamed on the basis of its amino acid sequence homology with human proinsulin (48%). A second, structurally distinct insulin-like peptide was found in serum and termed IGF-II. Insulin is considered to be a crucial anabolic hormone, which is produced and secreted to the plasma by the adult pancreas. The IGFs are important growth mediators, which are produced and secreted to the plasma by the adult liver, among other organs. In addition to these classical endocrine functions, the IGFs and possibly proinsulin are produced locally by many tissues throughout life, and have autocrine and paracrine functions *(44–47).*

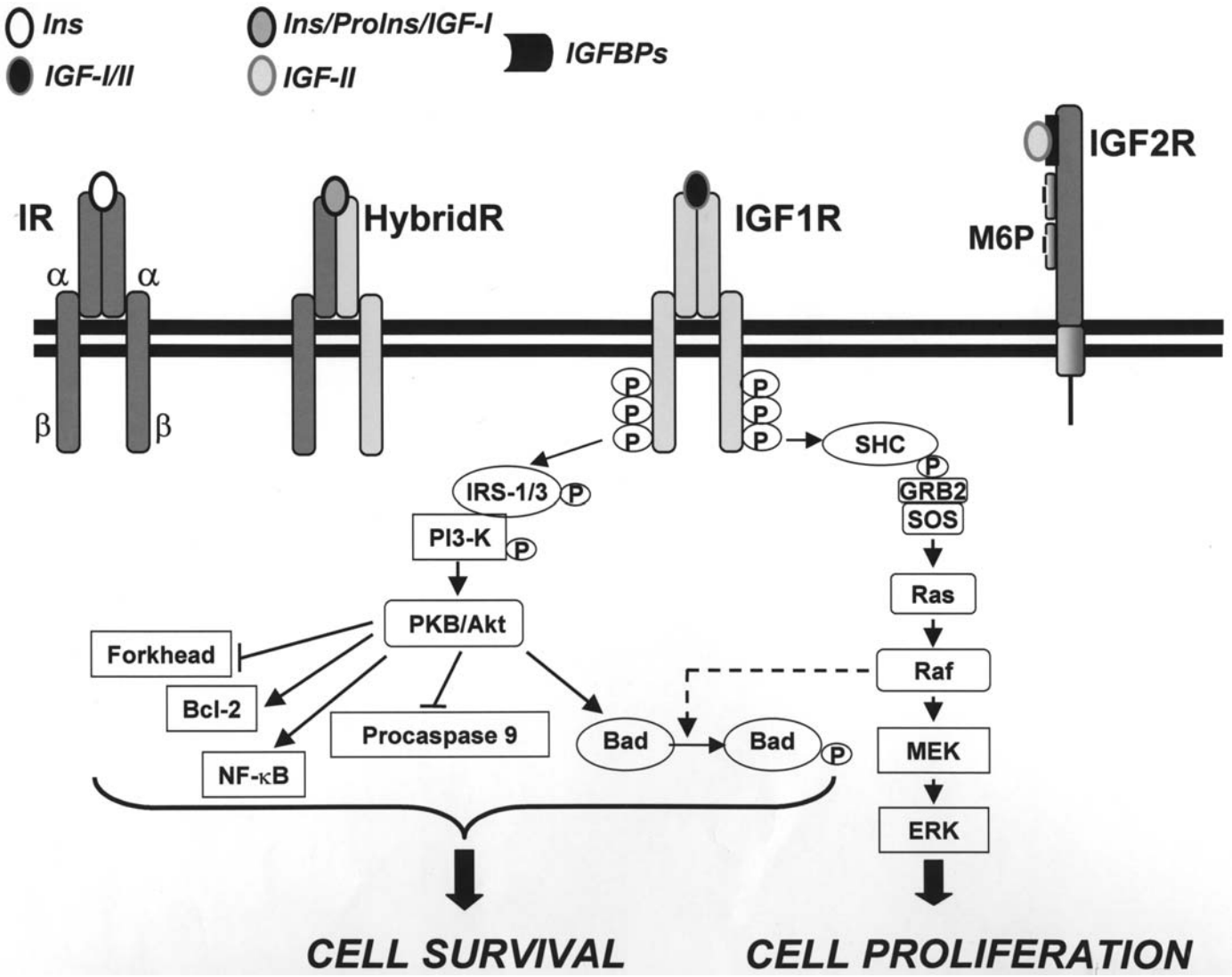


Fig. 1. Scheme of the insulin and insulin-like growth-factor signaling pathways. The insulin receptor (IR) and insulin-like growth-factor type 1 receptor (IGF1R) are $\alpha_2\beta_2$ heterotetramers. Hybrid receptor (HybridR) is composed of IR and IGFR hemimolecules. The extracellular glycosylated α subunits preferentially bind the ligands indicated (Ins, insulin; ProIns, proinsulin; IGF-I, insulin-like growth factor-I; IGF-II, insulin-like growth factor-II; M6P, mannose 6-phosphate). The IGF-binding proteins (IGFBPs) preferentially bind the IGFs. The β subunit of each receptor is a transmembrane polypeptide with a highly conserved tyrosine kinase catalytic domain. Binding of a ligand to its receptor results in receptor autophosphorylation on tyrosine residues and subsequent phosphorylation of intracellular proteins. Among these are the insulin-receptor substrates (IRS-1/3) that phosphorylate the regulatory subunit of phosphatidylinositol 3-kinase (PI3-K). This in turn activates PKB/Akt, a serine/threonine protein kinase that activates FKHR-L1 (Forkhead transcription factor), inactivates Bad by phosphorylation, and blocks procaspase 9, leading to the inactivation of apoptosis pathways, and thus, cell survival. Another signal-transduction pathway activated by insulin/IGF binding is the cascade that includes Ras, Raf, and MEK, which leads to activation of mitogen-activated protein kinases (ERK1/2) and cellular proliferation. For simplification, the intracellular pathways are represented for the IGF1R, but these pathways are shared by IR. The proposed scheme was summarized from refs. *29,31–33.*

The cellular actions of these factors, regardless of their endocrine, paracrine, or autocrine context, are mediated by binding to membrane-bound tyrosine kinase receptors (Fig. 1). The receptors were initially characterized during the 1970s. The classic receptors include the insulin receptor (IR) and the IGF type-1 receptor (IGF1R), both consisting of disulfide-linked heterotetramers conformed by two extracellular α subunits (binding activity) and two transmembrane β subunits (tyrosine kinase and signaling activities). IR and IGF1R show a high degree of sequence identity (60%). The IR binds IGF-I with 100-fold lower affinity than insulin. On the contrary, the IGF1R has the highest affinity for IGF-I (K_d 1 nM), although it is also capable of binding IGF-II and insulin with lower affinity (10- and 1000-fold, respectively). In certain tissues and developmental stages, the presence of hybrid IR/IGFR receptors has been reported in vivo. Hybrid receptors appear to be promiscuous, and interestingly, bind proinsulin—a factor with low affinity for IR that is barely able to activate IGF1R at physiological concentrations *(48–51).* The various receptors seem to be coupled to overlapping signaling pathways (Fig. 1). Further insight into the specific role of each receptor during development has been provided by the analysis of mutant mice *(52,53).* The possible basis of the selectivity of these factors in cellular responses is now beginning to be understood *(54,55).*

A completely different class of receptor is the cation-independent, mannose-6-phosphate monomeric IGF type-2 receptor (IGF2R). The IGF2R is a single-transmembrane protein that has no tyrosine kinase activity and binds preferentially to IGF-II (K_d range 0.1–1 nM). Its functions are related to traffic of lysosomal enzymes, as well as turnover and clearance of IGF-II in mammalian cells, and it has no known IGF-mediated signaling potential *(45,56,57).* Significantly, IGF-II does not bind to the IGF2R of non-mammalian species. Thus, any physiological function of this receptor in mediating IGF-II effects will have evolved after mammals diverged from other vertebrates *(58).* Homologs of the tyrosine-kinase receptors have been reported in other vertebrates and invertebrates *(41,59–66).*

Binding of IGFs to their specific receptors is further modulated by the IGF-binding proteins (IGFBPs), the last group of proteins in this system (Fig. 1). Six IGFBPs were initially characterized based on their ability to bind IGFs, with little or no affinity for insulin. More recently, putative new IGFBP family members (BPs 7–11) have been described *(45,67,68).* The actions of IGFBPs include serum carrier-protein functions (mainly IGFBP-3) and modulation of IGF actions. IGFBPs can either facilitate or inhibit IGF actions. The affinity of IGFBPs for IGF and their actions are modulated by several parameters, including the degree of IGFBP phosphorylation, glycosylation, differential localization, and proteolysis.

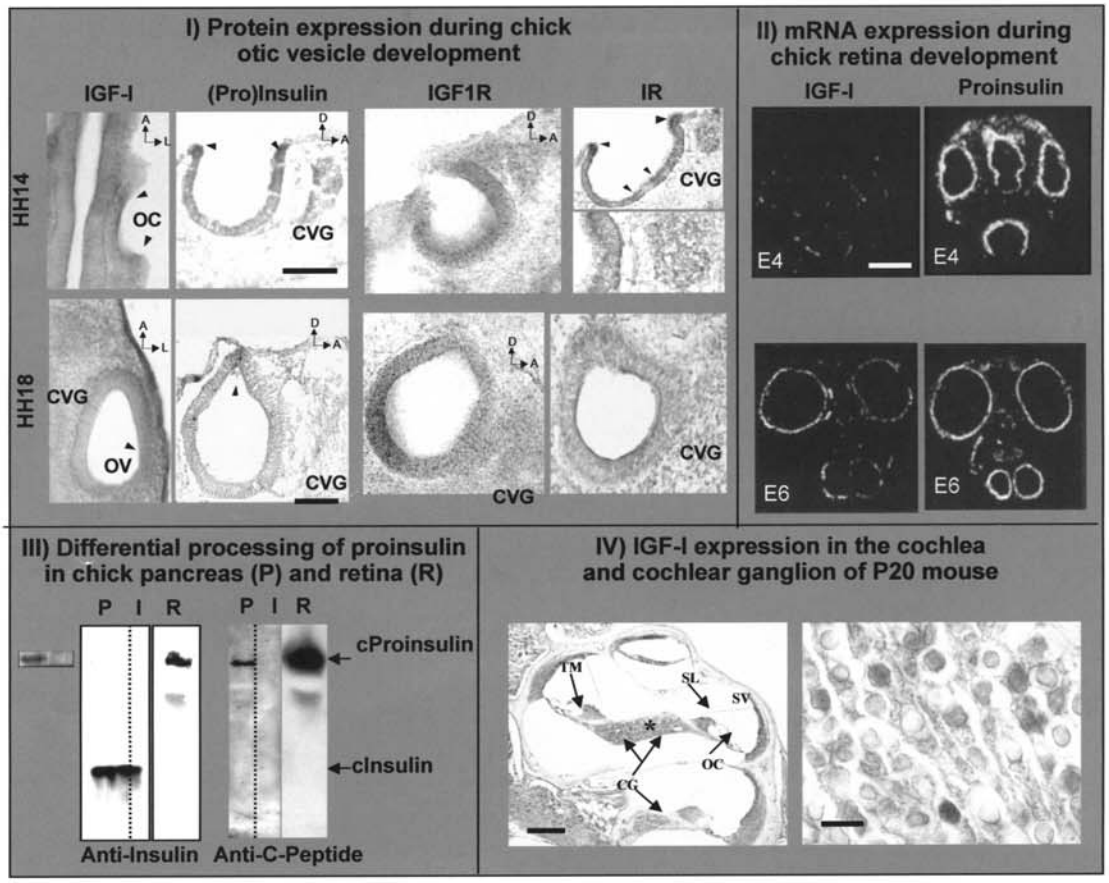
Neural Expression of the Insulin-Related Growth Factor System Elements

IGF-I and IGF-II are genuine neural growth factors. IGFs, their binding proteins, and the IGF1R are expressed in birds and mammals with remarkably conserved spatial and temporal patterns during development, maturation, and adult life (Table 1 and Fig. 2). IGF-II expression begins early in embryonic life, from neurulation onward, and is the predominantly expressed factor during early vertebrate development and organogenesis. IGF-II is localized in cells of non-neuroepithelial origin, which may provide an alternative potential source of this factor for the central nervous system (CNS) *(85,87,101).* IGF-I gene expression is weak during the first half of embryonic development, increases later on, and is downregulated shortly after hatching in chicks and during the first month of life in rodents *(85,114). igf-1* expression nonetheless remains high in certain areas of the brain that present plasticity (Table 1). For instance, in the postnatal rat cerebellar cortex, IGF-I is expressed exclusively in Purkinje cells. This expression is spatiotempo-

Table 1
Spatiotemporal Expression of IGFs and IGFBP1-6 During Nervous System Development and Postnatal Growth in Mammals and Birds

	IGF-I	IGF-II	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6
Early embryonic development	OV, CVG, BA, Ret	BA, NC, RP, Rho, NT, SNT, DRG, SN	n.d.	FP, RP, OP, NP	n.d.	n.d.	FP, SC	n.d.
Late embryonic development	OB, Hip, Ret, Tha, Cb, NB, PP, Tec	NT, Hip, Ret, Tec, Tha, Cb, CP, Men, Pit, Hyp	n.d	CP, Men, Pit, Cb, Ret	n.d.	FP, CP, Pit, Men, CPu, Tha, Hip	OB, Pit, Hip, Tha, Ms, Cb, Amy	TG
Early postnatal age	Pit, Cb, OB, CC, Hip, Tha, CPu, MO	Pit, SC, Men, Cb, MO, CP	Pit	Pit, Cb, Ret, OB, Tha	Pit	Pit, Men, Hip, OB	Pit, SC, OB, Cb, MO, Hip, Tha, Pons	Pit, SC
Adult animal	Cb, CC, Hip, Tha, OB, CP, Men	CP, Men, Cb, Str, Hip, Tha	n.d.	Amy, Pit, CP, Men, SC	CP, Men	CC, Hip, CP, Epe, Amy, BG, Tha, Men	CC, Hip, Tha, Cb, CP, Men, WM, SC, Epe	Hip, Tha, CP, OB, CN Men, Tha, CC, Cb, Pit, Amy, Pons,
Aged animal	Cb, CC, MO, Hip, Men	n.d.	n.d	n.d.	n.d.	n.d.	n.d.	n.d.

The table summarizes available data on mRNA expression of the IGFs and IGFBPs 1–6. It should be noted that the neuroanatomical distribution of the IGF axis proteins is broader than that of the mRNAs, as should be expected, considering their autocrine and paracrine mode of action. n.d., not determined. Abbreviations: Amy, amygdala; BA, branchial arches; BG, basal ganglia; Cb, cerebellum; CC, cerebral cortex; CN, cranial nerves; CP, choroid plexus; CPu, caudate-putamen; CVG, cochleovestibular ganglion; DRG; dorsal root ganglia; Epe, ependyma; FP, floor plate; Hip, hippocampus; Hyp, hypothalamus; Men, meninges; MO, medulla oblongata; Ms, mesencephalon; NB, nucleus basalis; NC, cranial neural crest; NP, nasal placode; NT, neural tube; OB, olfactory bulb; OP, otic placode; OV, otic vesicle; Pit, pituitary; PP, pineal primordium; Ret, retina; Rho, rhombencephalon; RP, Rathkes pouch; SC, spinal cord; SN, spinal nerves; SNT, spinal neural tube; Str, striatum; Tec; tectum; TG, trigeminal ganglion; Tha, thalamus; WM, white matter. Data summarized from refs. *69–110.*


I) Protein expression during chick otic vesicle development
IGF-I
(Pro)Insulin
IGF1R
IR
HH14
HH18
OC
CVG
OV
II) mRNA expression during chick retina development
IGF-I
Proinsulin
E4
E6
III) Differential processing of proinsulin in chick pancreas (P) and retina (R)
P I R
cProinsulin
cInsulin
Anti-Insulin
Anti-C-Peptide
IV) IGF-I expression in the cochlea and cochlear ganglion of P20 mouse
TM
SL
SV
OC
CG

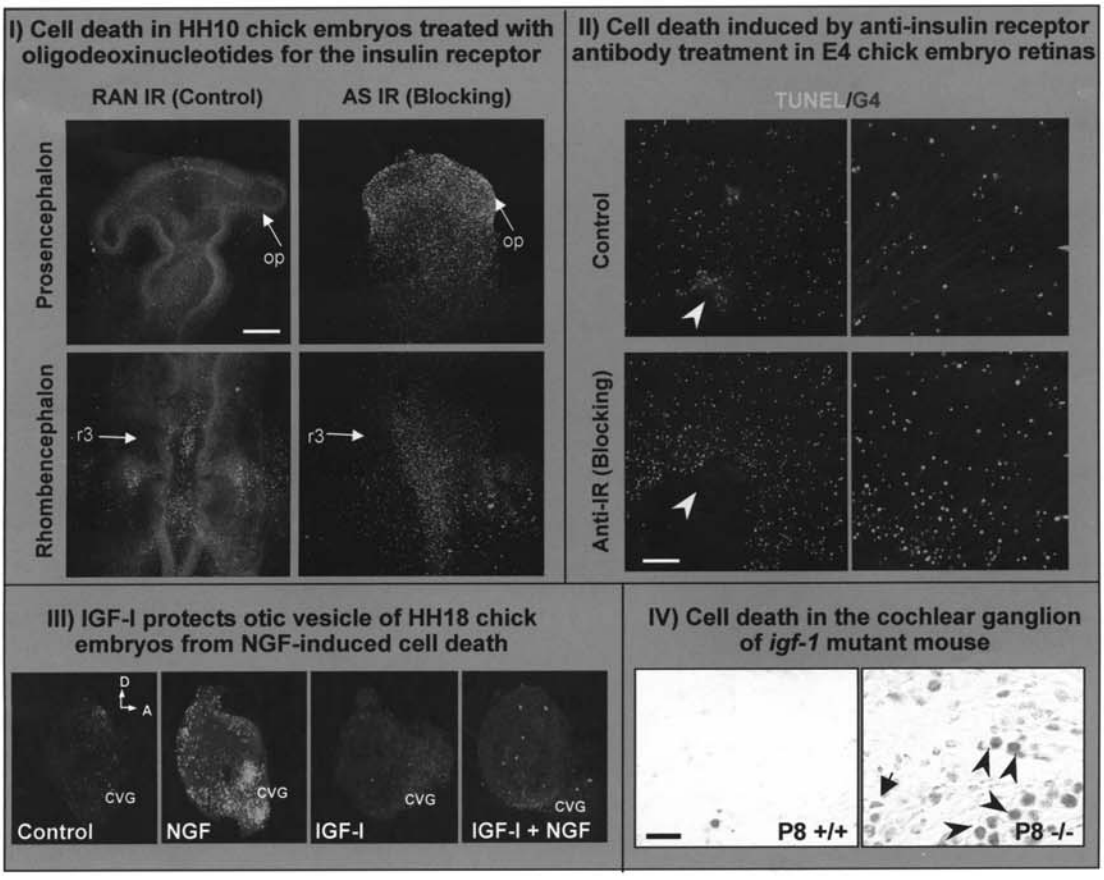

I) Cell death in HH10 chick embryos treated with oligodeoxinucleotides for the insulin receptor
RAN IR (Control)
AS IR (Blocking)
Prosencephalon
Rhombencephalon
op
r3
II) Cell death induced by anti-insulin receptor antibody treatment in E4 chick embryo retinas
TUNEL/G4
Control
Anti-IR (Blocking)
III) IGF-I protects otic vesicle of HH18 chick embryos from NGF-induced cell death
CVG
Control
NGF
IGF-I
IGF-I + NGF
IV) Cell death in the cochlear ganglion of igf-1 mutant mouse
P8 +/+
P8 -/-

Fig. 2. Expression of insulin-related growth factors and receptors in the chick and mouse. **(I)** Immunohistochemical localization in HH14 and HH18 chick embryo sections (HH, Hamburger and Hamilton stages). Arrowheads indicate areas of factor/receptor expression. A, anterior; D, dorsal; L, lateral; CVG, cochleovestibular ganglion; OC, otic cup; OV, otic vesicle. Bar-100 µm. **(II)** *In situ* hybridization in head sections of E (embryonic day of development) 4 and E6 chick embryos. The pseudocolor scale reflects the highest signal in red and the lowest in blue. Bar-2-mm upper panels, 3.2-mm lower panels. Reprinted with permission from ref. *112*. Copyright 2000, The Company of Biologists, Ltd. **(III).** Protein extracts were separated in 14% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted sequentially with anti-peptide C serum and anti-insulin serum. P, E15 chick pancreas extracts; I, purified pancreatic chicken insulin; R, E15 chick retinal extracts. Reprinted with permission from ref. *113*. Copyright 1998, Federation of European Biochemical Societies. **(IV)** IGF-I immunohistochemical localization in P (postnatal day) 20 mouse cochlea and cochlear ganglion of mice. The area marked by an asterisk is amplified on the right. CG, cochlear ganglion; OC, organ of Corti; SL, spiral limbus; SV, stria vascular; TM, tectorial membrane. Bars-600-µm left panel, 55-µm right panel. (Reprinted with permission from *111*. Copyright 2002, The Society for Neuroscience.)

rally coordinated with that of IGF1R and IGFBP-2 and -5 during stages of rapid synaptogenesis and dendrite formation *(74,89,115)*. IGFs are also expressed to a variable extent in brain tumors *(116–118)*. Concurrent with the widespread expression of the factors, IGF1R presents uniform, stable gene expression in all neuroepithelial-cell lineages during nervous system development. During postnatal differentiation, IGF1R expression remains selectively high in specific sets of sensory and cerebellar projection neurons that also express IGF-I *(73)*. In the adult rat brain, the presence of IGF1R, as for IGF-I, is also associated with structures that remain plastic into adulthood *(97,99,103,119,120)*. Schwann cells express both the IGF1R and IGF-I throughout postnatal development of the rat sciatic nerve *(121)*. During the normal aging process, which is associated with a reduction in hippocampal neurogenesis, there is a decrease in IGF-I levels and increased IGF1R expression *(99,122)*.

The other tyrosine kinase receptor of the system, IR, shows widespread regional expression

Fig. 3. Role of insulin-related growth factors in prevention of cell death in chick and mouse development. **(I)** HH10 chick embryos were treated for 8 h with insulin receptor anti-sense oligodeoxinucleotides or the corresponding control. Apoptotic cells were visualized by TUNEL in whole-mount embryos. Serial images were captured in a confocal microscope and compiled. The bright dots represent TUNEL-stained pyknotic bodies. op, optic vesicle; r3, rhombomere 3. Bar-200 µm. Reprinted with permission from ref. *44*. Copyright 2002, American Diabetes Association. **(II)** E2 chick embryos were treated for 2 d with anti-insulin receptor Igs or the corresponding control Igs. The retinas were processed as whole- mount. TUNEL staining (green) was employed to visualize the dead cells, and G4/Ng-CAM (red) was employed to localize the ganglion-cell axons. Bar-80 µm. Reprinted with permission from ref. *112*. Copyright 2000, The Company of Biologists, Ltd. **(III).** Otic vesicles were isolated from HH18 chick embryos, rendered quiescent, and cultured for 8 h in serum-free medium (Control), 4 nm NGF (NGF), 1 nm IGF-I (IGF-I) alone or in combination with NGF (IGF-I + NGF). Apoptotic cells were visualized by TUNEL. Compiled projections of the whole otic vesicle are shown. All otic vesicles have the same orientation: D, dorsal; A, anterior. CVG, cochleovestibular ganglion. Reprinted with permission from ref. *13*. Copyright 2002, The Company of Biologists Ltd. **(IV).** Apoptotic cell death in the basal turns of the cochlear ganglion of *Igf-1* mouse mutants was determined by TUNEL labeling of paraffin sections from postnatal d 8 (P 8) wild-type (+/+) and mutant (–/–) mice. Arrow and arrowheads indicate apoptotic Schwann and neuronal cells, respectively. Bar-30 µm. (Reprinted with permission from ref. *111*. Copyright 2002, The Society for Neuroscience.)

that matches that of IGF1R, even before the development of the pancreas *(29).* Insulin expression in the nervous system has been much less studied; yet embryonic and adult expression of insulin in the nervous system has been reported *(123–130).* Proinsulin mRNA is expressed in the neurulating chick embryo and the embryonic chick retina, and the translation product remains as unprocessed proinsulin (Fig. 2) *(44,112,129,131).* Finally, the IGF2R expression pattern appears to overlap with that of IGF-II *(132–134).* Recent studies of IGFs neural expression have focused on adult and aged animals (Table 1). Aging in mammals is characterized by alterations in brain structure and function, with decreases in the number and/or size of neurons in the cerebral cortex *(93).* In the brain, the cortical microvasculature and the meninges are important sources of IGF-I, and both present high IGF-I expression levels throughout life *(97).* Brain IGF-I mRNA distribution and levels do not change significantly with age, except in specific regions of the hippocampus, which show a decrease in the average expression per neuron *(86,93,97,99,122);* this coincides with the age-dependent reduction in the number of newly generated neuronal precursors *(135).* In the adult brain, the IGF1R expression pattern overlaps with that of IGF-I. IGF1R expression is associated to structures that remain plastic into adulthood, such as the dentate gyrus of the hippocampus, olfactory bulb, hypothalamic areas, and choroid plexus *(97,99,103,119,120).* IGF-I function in the adult brain relies on local sources, and can be imported from the periphery *(136);* indeed, serum IGF-I has positive effects on brain function and decreases amyloid-β levels *(137).*

IGF-I and -II expression appear to be regulated during neural injury. IGF-I is induced after brain damage within regions of cell loss *(72,78,103),* and IGF-I mRNA expression decreases in Purkinje cells that are undergoing apoptosis *(115,138).* A possible role has also been reported for IGF in preventing the death of motoneurons following sciatic-nerve injury and promoting peripheral-nerve regeneration *(139–141).* IGF2R expression is altered in response to lesions, suggesting a possible role for IGF-II/IGF2R in spinal cord physiology *(133,142).*

In the developing rodent CNS, glial cells and neurons synthesize IGFBPs locally, showing spatiotemporal expression patterns related to that of IGFs (Table 1) *(29,70).* Gene expression of IGFBP-1 and -6 occurs in astrocytes obtained from 1-d postnatal rat brain, whereas IGFBP-3 and -6 are expressed by cells of the oligodendroglial lineage that also produce IGF-I to support neuronal survival *(143,144).* IGFBP-2 is the predominant binding protein form in the human brain from 23 wk of gestation until 24 mo of postnatal age *(145),* and accumulates in human CNS tumors *(116).* IGFBP-5 is constitutively expressed in all brain regions postnatally, as well as in the glial cells and nerve tracts of the spinal cord *(72,98,103,107,146).* IGFBP-6-positive cells in the hindbrain and the periphery are associated with the coordination of sensorimotor functions in the cerebellum, which indicates an important role for the IGF-II/IGFBP-6 complex in the function and maintenance of these systems *(92).* IGFBPs expression is altered after nerve damage, suggesting a role in the neuroglial response following traumatic brain injury and neuronal death in the CNS *(72,78,82,96,103).* Finally, it is worth noting that studies of these molecules in the peripheral nervous system are scarce.

In summary, the insulin-related growth-factor system elements are expressed during development in most areas of the nervous system. As development proceeds and in adulthood, expression decreases and remains selectively associated with areas that maintain plasticity. Their presence in areas that retain their neurogenic capacity, together with the deregulated expression observed in situations of nervous system damage, suggest that the insulin-related growth factors are possible candidates for modulating neuroprotection and neurorepair in adulthood.

Actions of the Insulin-Related Growth-Factor System in the Nervous System

The vertebrate nervous system has a complex morphology that is the basis of its sophisticated functions. The cellular diversity and cytoarchitecture of the nervous system are generated largely during development by a delicate balance between proliferation, differentiation, cell death, migration, and maturation. Among the other molecules involved in correct development, maturation, and maintenance of the nervous system, a large number of reports demonstrate that the insulin-related growth factors are involved in neural development and physiology (summarized in Tables 2 and 3).

Insulin-related growth factors, especially IGF-I, appear to be involved in a number of processes throughout the life of a neural cell, from neural stem-cell maintenance to the synaptic modulation of neurons, and include increase in cell size, enhancement of proliferation, attenuation of cell death, increase of differentiation, and related processes such as axonogenesis or myelination, and finally, modulation of synaptogenesis and neurotransmission. These actions occur during development as well as in adult animals. As a dramatic example, homozygous partial deletion of the human IGF-I gene is associated with mental retardation and sensorineural deafness, as well as severe prenatal and postnatal growth failure *(260).* Although IGF-I is currently accepted as a neurotrophic factor whose value in neuroprotection has been considered in the treatment of neurodegenerative diseases *(261,262),* further studies are needed to evaluate the physiological roles and the potential of the other members of the system.

Cell Growth and Proliferation

Recent reports suggest that IGF-I is involved in regulating cell size during early postnatal development *(52,263). igf-1*-null mice show reduced size of the cochlear ganglia sensory neurons *(111).* This concurs with the increased cell size of hippocampal dentate gyrus neurons and medulla motoneurons in transgenic mice that overexpress IGF-I *(157,170,208).* In invertebrates, the insulin-related growth-factor system also participates in the control of cell size *(264,265).* Furthermore, insulin/IGF-I signaling controls cell growth and size during development through a cascade of intracellular events that are highly conserved in animals as diverse as nematodes, flies, and mammals *(38,41,266–270).* A striking observation, not yet defined in molecular and cellular terms, is the inverse correlation found among insulin-related growth-factor signaling and life span in vertebrates and invertebrates *(266,271).* In addition to cell size alterations, *igf-1*-null and -transgenic mice exhibit important changes in brain size that are caused by alterations in neuron growth and size as well as changes in the proliferation rate of neuronal precursors (Table 2). In vivo, transgenic mice that overexpress IGF-I show increased cell proliferation during early postnatal development in the cerebellum *(248).* Similarly, IGF-I infusion into the nose of newborn rats increases olfactory neuron proliferation *(210).* In specific areas of the CNS, the proliferative action of IGF is maintained throughout life. For example, IGF-I administration enhances proliferation in the dentate gyrus of the hippocampus *(252),* increases the reduced rate of proliferation shown by young IGF-I-deficient rats *(147),* and restores the proliferation rate in aged rats *(135).* The proliferative role of IGF is also important in restoring cell number after injury. Following olfactory axotomy, IGF-I administration at the lesion site enhances olfactory neuron proliferation *(272).*

Other elements of the system have also been implicated in the control of cell size and proliferation during nervous system development and early postnatal life *(52),* such as IGFBP-1 *(204,273)* and IGF-II *(274,275).* The use of organotypic and primary cell cultures has shown that insulin-related growth factors control the proliferation of neuronal precursors, neurons, and glial cells (Table 2), but the specific in vivo

Table 2
Insulin and IGFs Actions on Neural Processes

Process	Cell	Type (NS area)	Factor	References
Increase cell size	**CNS**	Neurons (Hip, MO, CC)	IGF-I	*157,170,208*
	PNS	Neurons (CG)	IGF-I	*111*
Enhance proliferation	**CNS**	Stem cells (Str)	IGF-I	*148*
		Neuronal precursors (Cb, Hip, Ret)	IGF-I, IGF-II, insulin	*135,147,151,165,182, 193,204,248,252*
		Glia (B, Cb, CC, FB, Ms)	IGF-I, IGF-II, insulin	*172,184,196,212,235, 238,249*
	PNS	Neuronal precursors (ONE, CVG, LSG)	IGF-I, IGF-II, insulin	*188,192,195,210,250,257*
		Schwann cells, OEG	IGF-I, IGF-II, insulin	*141,225,226,242*
Decrease apoptosis/ Enhance survival	**CNS**	Neurons (Cb, CC, Hip, Hyp, LMN, Ms, OMN, Ret, ScN, Str)	IGF-I, IGF-II, insulin	*113,128,140,144,164, 166,167,172,177,179, 180,189–191,197, 200–203,213,216,227, 230–235,240–242,251, 186,187,228,237,248*
		Oligodendrocytes (B, FB)	IGF-I, IGF-II, insulin	*163,206,244,249,255*
	PNS	Neurons (CVG, DRG, ONE, CG)	IGF-I	*112,195,214,215*
		Schwann cells (CG)	IGF-I	*112,154,159,168,169,198*
Differentiation	**CNS**	Neurons (Ret, Hip, SN, FB, CMN)	IGF-I, IGF-II, insulin	*147,152,173,175,181, 220,221,253,259*
		Glia	IGF-I, insulin	*218,236,249*
	PNS	Neurons (CG, ONE)	IGF-I	*112,195*
		Schwann cells (CG)	IGF-I, IGF-II, insulin	*160,161,225*
Neuritogenesis/ Axogenesis	**CNS**	Neurons (Cb, CC, FN, Hyp, ION, MO, MCN, OMN, ScN, Ret)	IGF-I, insulin	*95,155,170,171,183,207, 211,213,219,223,234*
Myelination	**CNS**	Neurons (B, CoC)	IGF-I	*157,204,246,247*
		Oligodendrocytes	IGF-I	*157,245,246,256*
	PNS	Neurons (CG, DRG, MCN)	IGF-I, IGF-II	*112,153,155,161*
		Schwann cells	IGF-I, IGF-II, insulin	*225*
Synaptogenesis	**CNS**	Neurons (Cb,CC, Hip, Hyp)	IGF-I	*156,158,174,207,208,254*
	PNS	Neurons (OC)	IGF-I	*112*
Neuromodulation	**CNS**	Glutamate (Cb, CC, Hip); GABA (Hip); Taurine (Ret); Noradrenaline (LMN); calcium channels (Cb, Pi, Ret)	IGF-I, insulin	*149,150,176,178,185, 194,199,209,217, 239,258,162,224*

Abbreviations: B, brain area not defined; Cb, cerebellum; CC, cerebral cortex; CG, cochlear ganglion; CoC, Corpus callosus; CMN, cranial motoneurons; CVG, cochleovestibular ganglion; DRG, dorsal root ganglia; FB, forebrain; FN, facial nerve; Hip, hippocampus; Hyp, hypothalamus; ION, Isthmo-optic nucleus; LMN, lumbar motoneurons; LSG: lumbosacral sympathetic ganglia; MCN, musculocutaneous nerve; MO, medulla oblongata; Ms, mesencephalon; OC, organ of Corti; OMN, ocularmotoneurons; ONE, olfactory neuroepithelium; OEG, olfactory ensheathing glia; Pi, pineal gland; Ret, retina; Str, striatum; ScN, sciatic nerve; SN, septal nuclei

Table 3
Survival Actions of Insulin and IGFs In Vitro

Cell Death Insult	Cell Type	NS Area	Factor	References
Serum and growth factor deprivation	Neurons	CC, Ms, Hyp, Cb, Ret,Tel, DRG, CVG	IGF-I, insulin	*12,13,144,167,172, 180,189,190,199, 214,216,222, 231–235,241,251*
	Schwann cells	ScN	IGF-I	*154,159,169*
Low density cultures	Neurons	Hip	IGF-I	*191*
Potassium deprivation	Neurons	Cb	IGF-I, insulin	*201,230*
BDNF deprivation	Neurons	Hip	IGF-I	*191*
FGF deprivation	Oligodendrocytes	FB	IGF-I, insulin	*244*
Hypoxia	Neurons	Hip	IGF-I	*242*
NO	Neurons	Hip, CC	IGF-I	*197,227,230*
High glucose	Neurons	DRG	IGF-I	*215*
	Schwann cells	ScN	IGF-I	*168*
Psychosine	Oligodendrocytes	B	IGF-I, insulin	*163*
TNF-α	Oligodendrocytes	B	IGF-I, IGF-II	*206,249*
NGF	Neuronal precursors	CVG	IGF-I	*12,13*
C_2-ceramide	Neuronal precursors	CVG	IGF-I	*12*
NMDA	Neurons	CC	IGF-I	*229*

Abbreviations: B, brain area not defined; Cb, cerebellum; CC, cerebral cortex; CVG, cochleovestibular ganglion; DRG, dorsal root ganglia; FB, forebrain; Hip, hippocampus; Hyp, hypothalamus; Ms, mesencephalon; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NO, nitric oxide; Ret, retina; ScN, sciatic nerve; Tel, telencephalon; TNF-α, tumor necrosis factor-α.

roles of each factor, receptors, and IGFBPs in cell growth and proliferation during development and adulthood are largely unknown. The levels and autocrine actions of IGFs in different nervous system tumors indicate that these factors may also regulate malignant neural growth *(116).*

Cell Differentiation

Insulin-related growth factors enhance the differentiation of specific sets of neural populations (Table 2). In vivo and in vitro studies demonstrate that insulin plays a significant role in cholinergic and GABAergic differentiation of the neurons in the developing avian retina, whereas IGF-I promotes the same action in the rodent hippocampus and septum *(181,185,221,276).* In the mouse, IGF-I deficiency causes decreased neuronal differentiation in the cochlear ganglion *(111,153)* and the dentate gyrus of the hippocampus, a defect reversed by the peripheral injection of the factor *(147).* Yet intracerebroventricular injection of IGF-I in aged rats does not reverse age-related decline of neuronal differentiation in this brain area *(135).* Stem cells derived from the olfactory bulb of *igf-1*-null mice do not differentiate normally *(277),* an observation that parallels the depletion of mitral neurons in the olfactory bulb of the *igf-1* null mouse *(278).* In the chick embryo, in ovo IGF-I blockage reduces the number of neurons *(175),* whereas IGF-I administration is able to generate new olfactory neurons from adult neuronal stem cells after olfactory nerve axotomy *(195).* Similarly, insulin intra-ocular injection in combination with FGF2 activates neuron production from Müller glial cells *(279).* In vitro treatment with exogenous IGF-I promotes differentiation

to neurons of mammalian stem cells, of teleost precursor cells, and of neuronal precursors in the chick otic vesicle (Table 2). Notably, developing neurons and adult neural progenitor cells share IGF-I responses. Fewer data are available on the in vivo capacity of IGFs to induce glial differentiation, but in vitro studies using primary glial-cell cultures suggest that these factors also promote differentiation and maturation of glial precursors (Table 2).

Neuritogenesis, Axonogenesis, Myelination, and Neuronal Plasticity

Once neuronal differentiation has occurred, dendritogenesis, axogenesis, and synaptogenesis are required to reach full function. In vitro studies and the few available in vivo data support that the insulin-related growth factors promote these actions (Table 2). Postnatal transgenic mice that overexpress IGF-I show an increase in motoneuron neuritic outgrowth *(170)*, whereas intracerebroventricular injection of IGF-I antisense oligonucleotides reduces the number and size of dendritic spines in cerebellar neurons *(207)*. Studies of nerve regeneration after lesion also demonstrate that insulin-related growth factors induce axonal repair in both the central and peripheral nervous systems *(261,280)*.

IGF-I also plays a significant role in myelination during nervous system development. Myelin staining, myelin basic protein, and proteolipid protein expression, as well as the percentage of oligodendrocytes and their precursors, are significantly reduced in all brain regions of developing *igf-1*-null mice, but are similar to controls in adult animals *(245)*. In contrast, in the postnatal mouse peripheral nervous system, IGF-I deficiency is associated with a sustained deficit in axonal myelination *(111,153)*. IGFBP-1 transgenic mice also show decreased myelination. In further support of the key role of IGF-I in myelination of the nervous system, IGF-I-transgenic mice show an increase in total brain myelin *(157,246,247)*. The possible roles of IGF-II and insulin in myelination are less often studied. IGF-II levels are increased in the brain of *igf-1*-null mice, and it may thus, partially compensate for IGF-I action on the myelination of the CNS *(245)*. IGF-II is also involved in the myelination of the musculocutaneous nerve after lesion, enhancing functional recovery *(155)*.

The study of mouse models with an IGF-I excess or deficit shows that this factor plays an important role in synapse establishment and maturation during nervous system development *(111,208)*. IGF-I is also involved in the remodeling and plasticity of synapses during adult life. Adequate IGF-I levels are needed in specific brain areas for the correct function of some neural tasks such as learning and memory *(158,207,281)*. IGF-I modulates synapse plasticity, modifying the number and structure of synapses *(156,174)* or regulating neuron firing and evoked field potentials *(282)*. Furthermore, IGF-I and insulin can regulate synapse activity by modulating neurotransmitter release and uptake, receptor endocytosis, and signal transduction of several neurotransmitters including glutamate, γ-aminobutyric acid (GABA), noradrenaline, and taurine (Table 2). Insulin and IGF-I also modulate ionic currents through L- and N-type calcium channels *(150,162,224)*.

In summary, many studies involving primary cell cultures and genetically modified animal models have shown that IGF-I and other insulin-related growth factors are essential for normal development and function of the vertebrate nervous system. Available data from invertebrates, although still quite limited, point in the same direction.

Neuroprotection from Physiological and Pathological Cell Death

In addition to the broad spectrum of actions summarized in the previous section, direct observations show that insulin-related growth factors support the survival of many types of neural cells at various developmental stages and in adult animals (Table 3 and Fig. 3). Insulin and IGFs are survival factors for neuroblasts in vivo and in vitro. During early post-

natal development, IGF-I-transgenic mice show a reduction in the apoptosis levels of granule-cell progenitors in the cerebellum *(164,248),* whereas IGF-1-knockout mice show increased apoptosis in early postnatal cochlear neurons *(111,153).* In the chick embryo, in ovo administration of IGF-I and IGF-II reduces naturally occurring cell death of lumbar motoneurons *(166,203,283),* IGF-I rescues chick ocular motoneurons *(213),* and insulin rescues chick retinal neuroepithelial cells and young neurons (Fig. 3) *(112,128).* Experiments using organotypic and primary cell cultures clearly corroborate the role of insulin and IGFs as survival factors that protect neurons and glial cells from cell death induced by different insults (Table 3). IGF-I protects chick otic vesicles from cell death caused by serum deprivation, nerve-growth factor, or exogenous ceramide (Fig. 3) *(12,13,284,285).* IGF-I—alone or in combination with other growth factors or neurotrophins—protects otic cells from ototoxic damage in vitro and in vivo *(286–288),* providing an example of the ability of early-development signals to act in neurorepair.

In vitro approaches have shed light on the intracellular signaling survival mechanisms activated by these factors (Fig. 1). Initial activation of the phosphatidylinositol-3 kinase/Akt is a common step in insulin-related growth factor signaling *(13,33,112,154,159,169,177,201, 222,241,289–292),* and downstream signaling diverges, depending on the cellular context and apoptotic stimuli. IGF-I is reported to prevent caspase-3 activation *(13,159,197,215, 229,230,242),* Jun N-terminal kinase activity *(159,284),* and *p53* transcriptional activity *(242),* to induce dephosphorylation of Bad *(177),* and to modulate Bax and Bcl-2 levels *(197,230).* One interesting aspect that merits further consideration is whether IGF-I prevents GSK3β-mediated neurodegeneration *(293).* In contrast, very little is known about the role and molecular mechanisms involved in the control of cell survival by other members of the insulin-growth factor family *(222,230).*

In recent years, considerable advances have been made in understanding the molecular and cellular basis underlying the specificity of action of each factor in particular cellular and developmental contexts. Nonetheless, the basis of the multiple actions of insulin-related growth factors remains an open question. We favor the hypothesis that many documented actions of the insulin-related growth factors can be considered to be permissive in nature, because neural cells undergo neural processes more efficiently in the presence of these factors. For instance, both insulin and IGF-I control glucose homeostasis, a central factor in cell welfare *(294,295).* It is also attractive to hypothesize that the anti-apoptotic activity of these factors is the basis of many of their multiple actions. Several lines of evidence support the hypothesis that IGF-I is a survival factor for neurons throughout adult life, and remarkably, in situations of neuronal damage. IGF-I is neuroprotective in Huntington's disease, and decreases brain amyloid-β levels *(137,296).* Yet IGF-I expression is low in Purkinje-cell-degeneration mice, a mutant mouse line characterized by increased cerebellar apoptosis *(115).* Severe hypoxia/ischemia induces a decrease in neuronal IGF-I levels concurrent with an increase in apoptosis in neonatal rat brain *(297),* and pretreatment with IGF-I attenuates ischemia-induced cell death of hippocampal *(227),* cortical *(240),* and striatal neurons *(179),* as well as of motoneurons *(202).* After nerve axotomy, IGF-I administration protects neurons *(186,187,203,237,272)* and Schwann cells *(198)* from cell death. Endogenous and locally administered IGF-II prevents motoneuron death after sciatic nerve axotomy *(140).* Other elements of the insulin-related growth-factor system exhibit changes in expression associated to central and peripheral nerve injury; hovewer, their role in neurodegeneration and neurorepair has not been studied in detail *(27).*

In summary, the attenuation of developmental and pathological cell death is a genuine action of the insulin-related growth factors. The extent to which this role leads to other observed actions requires new experimental approaches, including cell-death evaluation, that are specifically designed to pinpoint the

relative contribution of insulin-related growth factors to neural survival.

Perspectives and Conclusions

Most previous and current studies have focused on IGF-I, which is presently accepted as a neurotrophic factor. Indeed, the action of IGF-I in promoting neuron survival, axon growth, and myelination has prompted the use of recombinant human IGF-I in clinical trials for several neurodegenerative disorders that include amyotrophic lateral sclerosis (ALS), motoneuron disease, peripheral neuropathy, and adrenoleukodystrophy *(262,298).*

The dramatic impact of neurological diseases, including those caused by traumatic injury, genetic disorders, and age-related degeneration, affects not only the patients and their families, but society as a whole, and demands careful consideration of any possibility of attenuation. Therefore, the viability of IGF-I therapy has not yet been firmly established, and the potential of proinsulin and IGF-II are worthy of detailed study.

Acknowledgments

Research in the authors' lab is supported by grants from Dirección General de Investigación Científica y Tecnológica PM99-0111 to I. V.-N. and PM99-0094 to E. J. de la R. We are grateful to Flora de Pablo for helpful comments on this manuscript.

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