Chapter 9

REGULATION OF VERTEBRATE SENSORY ORGAN DEVELOPMENT: A SCENARIO FOR GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTORS ACTION

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1. INTRODUCTION

Sensorineural organs act as an interface between the nervous system and the world. Diverse sensorineural organs detect different kinds of environmental events using specialized sensorial receptor cells classified as chemoreceptor, thermoreceptor, mechanoreceptor, and photoreceptor cells. Chemoreceptor cells are associated with the senses of taste and smell and are sensitive to chemicals in the environment. The taste buds of mammals detect dissolved food molecules and are located primarily on papillae of the tongue[1]. The olfactory epithelium is designed for recognition of volatile molecules and is located in the nose in terrestrial vertebrates. The olfactory cells present olfactory cilia that specifically respond to a large number of different chemicals in the air, including as many as 1000 types of different

smells in humans[1,2]. Thermoreceptor cells are associated with the sense of touch. In mammals, changes in temperature are detected by cold, warmth and pain thermoreceptors[1]. Mechanoreceptor cells respond to many different stimuli, such as sound, touch, pressure, gravity, stretch, or movement. Touch receptors are located in the skin and perceive touch, pressure, and pain[1]. Other mechanoreceptors include those located at the base of hairs and bristles that respond to motion and inner ear receptor cells that sense sound and balance[1]. Finally, photoreceptor cells located in the retina use pigments to absorb and detect light. The sensitive rods are specialized in low-intensity light detection, while the cones work better with daylight and are able to analyze the different light wavelengths responsible for the colors[1]. Upon specific activation, all types of receptor cells generate changes in membrane potentials that initiate the transmission of information to the brain that then reads this information and generates the adequate body responses.

In this chapter we summarize the available studies concerning the development of sense organs under physiological and pathological conditions, with respect to GH/IGF action.

2. DEVELOPMENT OF SENSORINEURAL ORGANS

The vertebrate sense organs arise at early stages of embryonic development and have distinct developmental origins. A variety of extrinsic signals control sensorineural development, including members of different families of cytokines, and growth and neurotrophic factors. The insulin-like growth factor (IGF)/growth hormone (GH) axis plays a central role in the development of the sensory organs, where these proteins and their receptors are widely expressed (Table 1)[3-26]. A detailed description of the elements of the IGF system and signaling pathways is provided in other chapters of this book[27, 28].

GH availability, the expression of its receptor (GHR) and some actions on the sensorineural organs have also been reported, however, there is less information available than for the IGF system.

	IGFI	*IGFIR*	*IR*	*GHR*	*IGFII*	*BP-1*	*BP-2*	*BP-3*	*BP-4*	*BP-5*	*BP-6*
Early Embr. Dev.	OV CVG Ret Lens	OlfP Ret	OB	OpV	Ret	n.d.	OtP NP	n.d.	n.d.	n.d.	n.d.
Late Embr. Dev.	OB Ret	OE Ret	OB	Ret Lens	Ret	n.d.	Ret	n.d.	n.d.	OB	n.d.
Early Post. age	OB Ret Cp Corn	OE Ret Lens Conj Corn	n.d.	n.d.	n.d.	n.d.	Ret OB Corn Iris Conj Scl	Cp Chr Corn	OB Cp Chr Corn	OB Ret Cp Chr Corn	Ret Cp Chr Corn
Adult	OB Ret Cp Corn	OE Ret Lens Conj Corn	n.d.	n.d.	n.d.	n.d.	Corn Iris Conj Scl	Cp Chr Corn	BG Cp Chr Corn	Ret Cp Chr Corn	OB Ret Cp Chr Corn

Table 1. Expression of factors, their receptors and BPs during sensory organ development and postnatal growth. This table summarizes available data on mRNA expression of IGF-I, IGF-1R, IR, GHR, IGF-II and IGFBPs 1-6 (refs. 3-26). **Vision**: Chr, chromatophores; Conj, conjunctiva; Corn, cornea; Cp, choroid plexus; OpV, optic vesicle; Ret, retina; Scl, sclera; **Inner ear**: CVG, cochleovestibular ganglion; OtP, otic placode; OV, otic vesicle; **Olfaction**: NP, nasal placode; OB, olfactory bulb; OE, olfactory epithelium; OlfP, olfactory placode. Emb, embryonic; Dev, development; Post, postnatal; n.d., not determined.

Many sense organs have a placodial developmental origin[29,30]. The inner ear and the olfactory system arise from the otic and olfactory placodes, respectively. Placodal development starts as discrete ectodermal thickenings that, through complex morphogenetic and neurogenetic processes, generate the adult structures. Furthermore, placodes can modulate the development of related structures. The olfactory placode is essential for normal forebrain development[30] and the olfactory and otic epithelia induce chondrogenesis in the surrounding mesenchyme, providing a protective, rigid structure for these sensorineural organs, which also contributes to the structural scaffold of the head. The optic lens also has a placodial origin and it is essential for normal development of the retina and other adjacent structures, such as the iris, the ciliary body and the overlying cornea[30-34].

Effect	Neural Target	Factor
Increase cell size	Neurons (CG, OB)[36, 53]	IGF-I
Enhance proliferation	Neuronal precursors (Ret)[37-39]	IGF-I, IGF-II, Insulin
	Stem cells (OB)[64]	IGF-I, Insulin
	Neuronal precursors (CVG, ONE)[19, 40-43]	IGF-I, IGF-II, Insulin
	Schwann cells (OEG)[44,45]	IGF-I, IGF-II, Insulin
Decrease	Neurons (OMN, Ret)[46-52]	IGF-I, IGF-II, Insulin
apoptosis/enhance	Neurons (CVG, ONE, CG)[42,54,63,68,69]	IGF-I
survival	Schwann cells (CG)[36,55-59]	IGF-I
Differentiation	Neurons (Ret)[38,60-62]	IGF-I, IGF-II, Insulin
	Stem cells (OB)[64]	IGF-I, Insulin
	Neurons (CG, ONE, OB)[36,42,64]	IGF-I
	Schwann cells (CG)[45,65,66]	IGF-I, IGF-II, Insulin
Neuritogenesis/	Neurons (ION, OMN, Ret)[15,24,48,67]	IGF-I, Insulin
Axogenesis	Neurons (OB)[19,53,70,75]	IGF-I, IGF-II
	Olygodendrocytes (OB)[53,70,75]	IGF-I
Myelination	Neurons (CG)[36,71]	IGF-I, IGF-II
	Schwann cells[45]	IGF-I, IGF-II, Insulin
	Olygodendrocytes (OB)[53]	IGF-I
Synaptogenesis	Neurons (OC)[36]	IGF-I
Neuromodulation	Taurine (Ret)[73]	IGF-I, Insulin
	Calcium channels (Ret)[72,74]	IGF-I, Insulin

Table 2. IGFs actions on the sense organs. **Vision:** ION, Isthmo-optic nucleus; OMN, ocularmotoneurons; Ret, retina; **Inner ear:** CG, cochlear ganglion; CVG, cochleovestibular ganglion; OC, organ of Corti; **Olfaction:** OB, olfactory bulb; OEG, olfactory ensheating glia; ONE, olfactory neuroepithelium.

During the last decade, progress has been made in the understanding of the molecular basis of sensorineural organ development[5,16,29,30,35], as well as in the role of IGFs in neural development. IGFs are fundamental for the development, maturation, and functionality of the nervous system, being involved in the regulation of processes such as cell proliferation, survival, and differentiation[3]. Studies on sensorineural development (Table 2)[12,16,19,21,36-75], although less abundant, point in the same direction.

Diseases and Syndromes	Sensory System Dysfunction	GH/IGF axis Dysfunction
Idiopathic late onset cerebellar ataxia[75]	Reduction of olfactory function	Reduced IGF-I levels
Amyotrophic lateral sclerosis[75]	Reduction of olfactory function	Reduced IGF-I levels
Turner's syndrome[78]	Otitis media Sensorineural hearing loss	Reduced IGF-I levels
Laron's syndrome[80,81]	Deafness Retinitis pigmentosa	GHR defects Abnormalities of GH signal transduction Primary defects on IGF-I synthesis or secretion
Usher's syndrome[83]	Deafness Retinitis pigmentosa	n.d.
Igf-1 mutations[76,77,82]	Deafness	Elevated GH secretion Undetectable serum IGF-I Normal serum IGFBP-3
IGF1R mutations[84]	n.d.	Decreased IGF1R levels Reduced affinity for IGF-I Increased concentrations of IGF-I and IGFBP-3 Decreased concentrations of IGFBP-2
Leber congenital amaurosis syndrome[85]	Abnormal eye movement Vision defects	GH insufficiency
Septo-optic dysplasia[86]	Optic nerve hypoplasia and pituitary dysfunction	GH insufficiency

Table 3. Clinical alterations associated with defects in the GH/IGF axis.This table summarizes human diseases and syndromes that present a dysfunction of the sense system associated with alterations in the GH/IGF axis[75-86]. n.d., not determined.

Alterations in the GH/IGF axis are causal to defects in the development of human sense organs (Table 3)[75-86]. The study of animal models that present altered levels of expression of one or more elements of the GH/IGF axis has, indeed, provided further insight into some of these human diseases and syndromes[28,71]. An understanding of the regulation of sensorineural development may contribute to the elucidation of the origin or progression of human diseases such us blindness or deafness, and to the development of novel therapeutic strategies.

3. THE VISUAL SYSTEM

Although the retina is a part of the central nervous system (CNS), because of its sensorial function and peripheral location it is considered as a sensorineural organ. The sensorial attributes reside in the innermost layer of the eye, the neuroretina. The neuroretina and the adjacent pigmented epithelium, together with part of the iris and ciliary body, are derived from the neuroepithelial wall via optic vesicle and optic cup. The lens, and to a lesser extent the cornea, is generated from the placoda formed in the overlying surface ectoderm (Fig. 1A). The vertebrate neuroretina is a complex neuronal network comprising six neuronal types: the rod and cone photoreceptors, responsible for the absorption of various wavelengths of light; the bipolar, horizontal, and amacrine interneurons, which integrate photoreceptor information; and the retinal ganglion cells, which transmit this information to the brain for cognitive processing. In addition to these neuronal types, Müller glial cells also originate within the retina. The outer most layer of the eye is the sclera, which at the front of the eye is transformed into the transparent cornea that permits light rays to enter the eye. The middle layer includes the iris, the ciliary body, and the choroid.

GH gene expression occurs in extrapituitary tissues prior to, and even after, the organogenesis of the pituitary gland[87-90]. GH immunoreactivity is detected early in development in, among other nervous system locations, the chick otic and optic vesicles. Later in development, but still prior to the differentiation of pituitary somatotrophs, the chick neuroretina, the pigmented epithelium, and the epithelial lens fiber cells show intense GH immunoreactivity[88,91]. The distribution of the GH receptor mirrors that of GH. Since GH is absent from the circulation of early chick embryos [87,88], these observations suggest that extrapituitary GH expression has an autocrine/paracrine role during early embryogenesis, in particular in the development of the ear and the eye[87-90,92]. The presence of GH and its receptor correlates with a suggested involvement of GH in the regulation of ocular development by acting on the intraocular melanocortin system in the chick[91] or inducing retinal angiogenesis. Indeed, GH deficiency in humans is associated with reduced retinal vascularization[93], whereas exogenous GH promotes retinal angiogenesis[94].

The actions of GH during embryonic development could be mediated by other growth factors, particularly IGF-I[95,96]. The widespread expression of mRNAs for IGF-I, IGF-1R, and IGFBP-2 to IGFBP-6 in specific histological layers of the retina, choroids, ciliary body and cornea in the rat suggests specific roles of the IGF axis in the eye[3,9,10] (Table 1). The developmental expression of most IGF family members has been described in the eye of birds[3,5], mammals[3] and fish[6,7] when proliferation and

differentiation of neuroretinal cells occur. IGF is also involved in lens differentiation. The presence of IGF-I mRNA in ocular embryonic tissues suggests an autocrine/paracrine function of the IGFs.

In addition to GH/IGF-I axis involvement in normal development, its deregulation has pronounced physiological effects such as dwarfism (associated with low levels of GH), gigantism, and acromegaly (associated with high levels of GH)[96,97]. More specifically in the visual system, Leber congenital amaurosis syndrome is associated with short stature, growth hormone insufficiency and vision defects[85]. Septo-optic dysplasia (SOD), a disorder of multifactorial etiology that includes gestational diabetes, is characterized by optic nerve hypoplasia with pituitary dysfunction[86]. Children with SOD may manifest a variety of visual and/or physical symptoms that range from mild to severe[86, 98-100]. A case of a 14-year-old boy with optic hypoplasia and pituitary dwarfism due to a complete deficiency in GH has also been reported[101]. A rare case of GH and gonadotropin deficiency associated with dysmorphic features has been reported in a 16-year-old boy with clinical characteristics that included left anophthalmia (absence of the left eye), microphallus, bilateral cryptorchidism, and mental retardation[102]. All of these features are attributed to the hypothalamic dysfunction and are very similar to the features of septo-optic dysplasia, but mutation analyses revealed no mutations or polymorphisms in the SOD associated gene *HESX1*[102].

Vascularization of the retina normally occurs during fetal development, with little or no vascularization after birth[103]. A role for GH in normal retinal vascular development has been suggested because children with congenital GH deficiency have reduced retinal vascularization[93]. GH may exert its effects through circulating or locally produced IGF-I since the lack of IGF-I during the early neonatal period is associated with lack of vascular growth and retinopathy of prematurity, a blinding disease initiated by lack of retinal vascular growth after premature birth[104,105]. IGF-I probably influences angiogenesis and the development of retinal neovascularization through interaction with locally produced factors such as vascular endothelial growth factor (VEGF), by acting as a permissive factor for maximum VEGF stimulation of angiogenesis[104]. IGF-I above a specific threshold level is necessary for maximum VEGF activation of the MAPK and Akt pathways, pathways important for endothelial cell proliferation and survival[104]. However, ischemia-induced retinal neovascularization is only partially suppressed in transgenic mice expressing a GH antagonist gene[106].

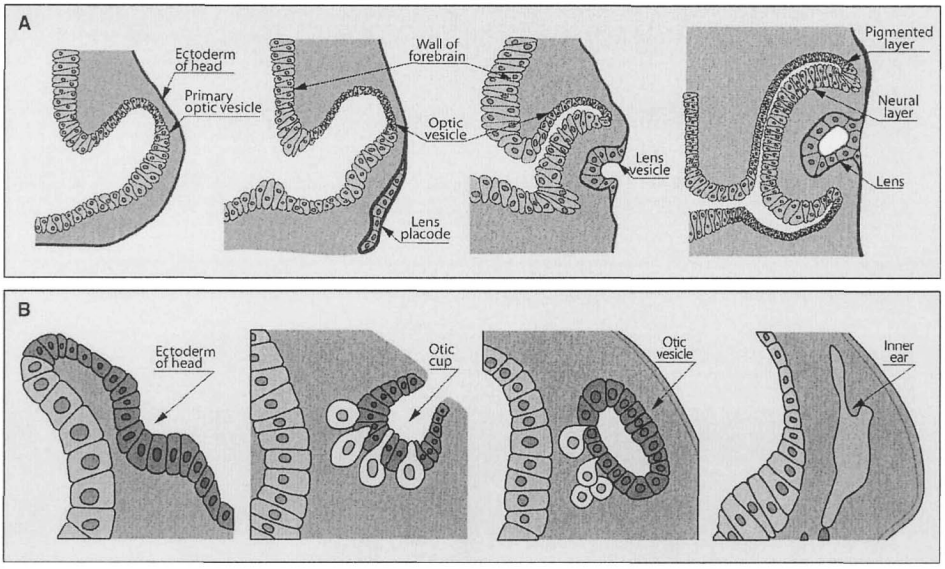

Figure 1. (**A**). Neuroretina development. (**B**). Inner ear early development.

4. THE OLFACTORY SYSTEM

Most elements of the olfactory system are generated from the most rostrally placed placodes, the olfactory placodes. In mammals, these placodes give rise to four types of neurons: olfactory, sense or vomeronasal, nervus terminalis, and migratory gonadotrophin-releasing hormone (GnRH) neurons[107], plus other cell types such as receptor cells or glial and Schwann cells. The olfactory neurons follow distinct patterns of cell differentiation and migration and, *pari pasu* the nasal epithelium involutes and induces the chondrogenic capsule in the surrounding mesenchyme. A characteristic of GnRH neurons is that they are generated outside the CNS and migrate toward it[30,108,109]. Also remarkable is the fact that olfactory sense neurons are renewed from progenitor cells present in the olfactory neuroepithelium in adult mammals[110,111]. Therefore, the olfactory system is a source of adult stem cells[112]. The axons of the olfactory sensory neurons are surrounded by a special type of glial cell, called olfactory ensheathing glia, which are also derived from the olfactory placode[113-116]. The olfactory epithelium has intrinsic growth factors that regulate development and can support the genesis and survival of new neurons[18]. The members of the IGF system, in particular IGF-I, are expressed in the olfactory system cells and can influence neuronal generation, survival, and/or differentiation (Tables 1 and

2). Expression of both IGF-I and its high-affinity receptor IGF-1R decreases and/or becomes more restricted to specific cell types in the olfactory structures with age in different vertebrates[16,17,24]. However, it is important to note that the olfactory bulb is one of the areas in the CNS that retains relatively high expression of IGF-I and the IGF-1R, with this higher expression generally being associated with areas of adult neurogenesis[3]. Regarding the function of IGFs during olfactory system development, expression patterns in the olfactory bulb suggest a role for IGF-I from early neurogenesis onwards and, accordingly, *Igf-1* knockout mice present severe alterations in the formation of the mitral cell layer of the olfactory bulb, as well as altered morphology of radial glia[64].

5. THE GUSTATORY SYSTEM

The development of the vertebrate taste system has been relatively understudied compared with other sensory systems. Tongue development starts when the first pharyngeal arch forms a swelling called the median tongue bud, shortly afterwards another pair of lateral swellings, the distal tongue buds or lateral lingual swellings, is formed, which rapidly overgrow the median tongue bud. These structures continue to grow throughout development and form the anterior two-thirds of the tongue. From the second pharyngeal arch develops a midline swelling called the copula that is later overgrown by the hypopharyngeal eminence, which gives rise to the posterior one-third of the tongue. The hypopharyngeal eminence expands mainly by the growth of the endoderm of the third pharyngeal arch, with a small contribution from the fourth pharyngeal arch to the most posterior aspect of the tongue[117]. The capacity of the pharyngeal endoderm to generate taste buds is probably determined by the end of gastrulation, but the molecular bases of this process are yet to be elucidated. The sensory afferent axons from the VIIth, IXth, and Xth cranial nerves invade the lingual epithelium before taste bud differentiation and it has been proposed that innervation may play a role in taste buds differentiation[118]. The neurotrophic factors brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) participate in the innervation of the developing tongue in mouse and humans. It has been reported that mice with deficits in BDNF or NT-3 present gustatory or somatosensory alterations, respectively[119-122]. In addition, alterations in size, number and morphology of gustatory papillae and taste buds present in BDNF null mutant mice demonstrate the neural dependence of developing taste organs[123].

To our knowledge, there are no studies on the actions of the GH-IGF axis on the development of the gustatory system.

6. THE TACTILE SYSTEM

The sense of touch allows the perception of stimuli such us contact, pressure, temperature or pain. Its sensory organ is the skin. The perception of stimuli is carried out by skin specific sensory mechanoreceptors, thermoreceptors and nociceptors located throughout the layers of the skin (epidermis, dermis and hypodermis). For example, Meissner's corpuscles sense the onset and end of continuous light pressure, Pacinian's corpuscles sense firm pressure, Ruffini's corpuscles and Krause's corpuscles respond to changes in temperature and pressure on the skin. There are four types of mechanoreceptive afferent neurons that innervate the skin and respond to cutaneous motion and deformation in different ways[124]. The association of specialized receptor cells with peripheral terminals of sensory axons forms the sensory receptors in the skin. The specificity of the receptor is determined by the subtype of transducer that is stimulated and by the structure of the receptor that surrounds each of these nerve terminals[124, 125]. The development of the innervating sensory nerve cell occurs *pari pasu* with that of the receptor cell. The neurons promote receptor cell differentiation, which in turn provide neurotrophic support to the sensory neurons. The signals that mediate these actions are not known in detail, but the influence of neurotrophic factors on the development of the tactile system has been confirmed by the study of transgenic mice lacking neurotrophins or their receptors[126-128].

Actions of the IGFs in the regulation of tactile corpuscles development or in the perception of the stimuli have not yet been reported.

7. THE AUDITORY AND VESTIBULAR SYSTEMS

The vertebrate inner ear is derived from a thickening of the head surface ectoderm, adjacent to rhombomeres 5 and 6, named the otic placode that invaginates and pinches off the ectoderm to form the otic vesicle or otocyst. Figure 1B shows the early stages of vertebrate inner ear development. The otic vesicle is a transient structure that undergoes multiple morphogenetic movements and developmental changes associated with cell proliferation, differentiation and cell-death. This results in the ear labyrinth: the cochlea,

the utricle and saccule, and the semicircular canals, each containing their corresponding sensory organs[35,129,130] with the mechano-transducing hair cells and the neurons that connect them with the central nervous system. Local mechanical perturbations are transduced by hair cells into synaptic potentials, which elicit the activation of auditory (cochlear) and vestibular neurons, that project towards the homonymous central nuclei, the first input station of the auditory pathway. Auditory neurons inform the brain of the intensity and spectral properties of sound and vestibular neurons carry information about position, velocity and acceleration. Generation of otic neurons is a sequential process. First, otic neurons are specified in the otic epithelium, neuronal precursors then delaminate to form the cochleo-vestibular ganglion (CVG), where they proliferate and differentiate. The CVG neurons project extensions back to innervate the vestibular and cochlear (auditory) sensory epithelium.

Diffusible factors like fibroblast growth factors, IGF-I and the nerve growth factor family of neurotrophins are locally synthesized during development and elicit a network of interconnected signaling pathways that finally instructs the cell to proliferate, die or differentiate. The elements of the IGF system are expressed in the early developing chicken inner ear[3,14] (Table 1). GH immunoreactivity has also been detected during early development of the chicken otic vesicle[88,90-92]. In the developing vertebrate inner ear, IGF-I acts as a survival and growth factor [135]. IGF-I is also expressed during maturation of the rodent auditory system and in adult hair cells[131,132]. During the early postnatal period of mouse inner ear development, from postnatal day (P)5 to P20, IGF-I is expressed in the Organ of Corti and cochlear ganglia[36]. The cochlear and vestibular ganglia also express insulin, IGF-II and their receptors (Fig. 2A). Recently, the analysis of a human fetal cochlear cDNA library indicated the presence of IGF-I and IGFBP-1, -3 and –5. Analysis of gene expression profiles of the rat cochlea demonstrated the presence of IGF-II and IGFBP-2 and -6[133,13]. Studies on primary cell cultures and genetically modified animal models have shown that IGF-I is essential for the normal development and function of the vertebrate inner ear[135] (Table 2). Endogenous IGF-I is essential for generation of the CVG in chicken embryos, with the blockade of IGF-I actions being associated with an increase in cell death, a reduction in cell proliferation and a reduction in the levels of expression of neuroblasts and neuronal markers[140].

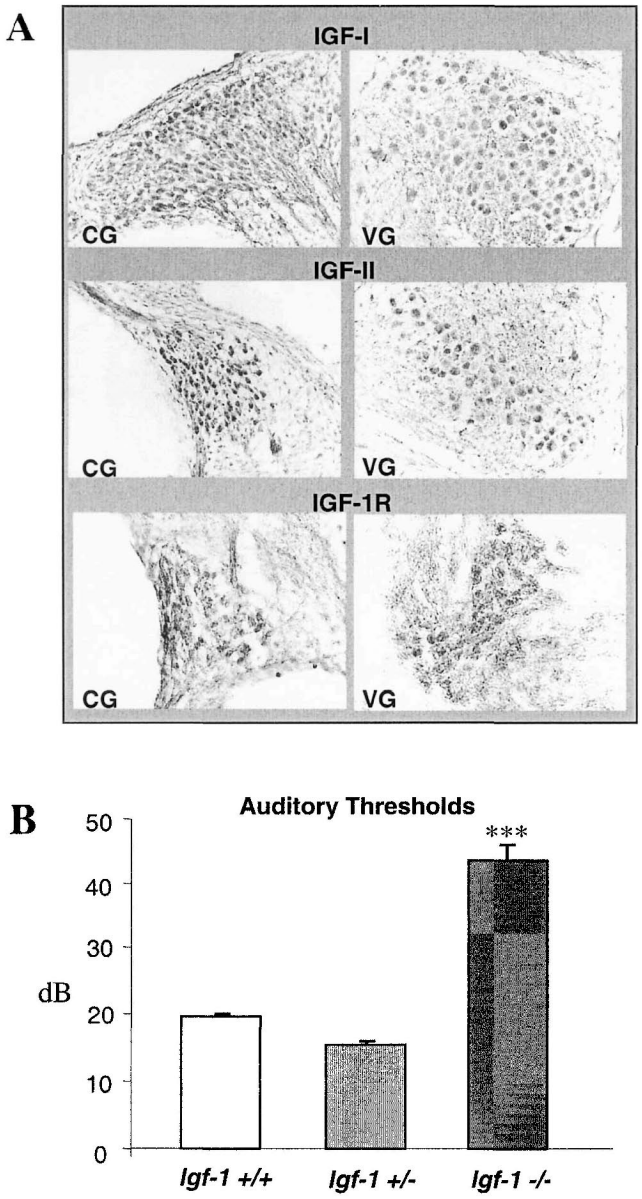

Figure 2. **A.** Expression of insulin-related factors and receptors in the cochlear and vestibular ganglia in P5 mice. Immunolocalization of IGF-I, IGF-II, and IGF1R was positive in the cochlear (CG) and vestibular ganglia (VG) of P5 mouse. Microphotographs show representative 25 μm paraffin sections from at least four mice studied in three different assays. Scale bars, 50 μm. **B.** Auditory thresholds of wild type (n=25), heterozygous (n=30) and knockout *Igf-1* mice (n=7) at P30. Statistical analysis reveal that the *Igf*-1 deficient (-/-) mouse has a two-fold increase in auditory thresholds (*** P<0,001) compared to control (+/+) and heterozygous (+/-) mice.

We have explored the signaling mechanisms that mediate IGF-I proliferative responses in otic cells. IGF-I binding to IGF1R increases the levels of inositol lipid mediators, activates the Raf/MAPK cascade, and induces the expression of the transcription factor AP-1 and proliferating cell nuclear antigen (PCNA)[40,43,136-138]. Anti-apoptotic effects of IGF-I are mediated by the activation of the Akt/protein kinase B pathway and modulation of the levels of the pro-apoptotic lipid mediator ceramide[139].

Interestingly, the early actions of IGF-I on cell proliferation are mimicked by other factors of the family, insulin and IGF-II, but later actions on cell differentiation are specific to IGF-I[43,140]. Accordingly, during the mouse inner ear postnatal period of maturation, from birth to P20, the neurons of the cochlear ganglia become strictly IGF-I dependent and its deficit causes retarded maturation and decreased cell survival[36,71]. Moreover, IGF-I deficiency causes decreased neuronal differentiation in the mouse cochlear ganglion, a sustained deficit in the cochlear nerve and ganglia myelination, and aberrant synaptogenesis at the Organ of Corti, suggesting that IGF-I is required to reach full auditory function[36,71]. We do not yet know whether the alterations observed in cochlear neurons of *Igf*-1 null mice could compromise mid-term survival of hair cells of the organ of Corti. We have confirmed by using auditory brainstem response tests that *Igf-1* knockout mice at day P30 have an increase in auditory thresholds compared to control (*Igf-1*$^{+/+}$) and heterozygous (*Igf-1*$^{+/-}$) mice[135] (Fig. 2B). These results confirm the key role of IGF-I in the development and maintenance of auditory function. Indeed, a deficiency in IGF-I results in sensory-neural deafness in humans[76,77,82] (Table 3), but a partial deficiency in IGFR1 is not associated with deafness in humans, although no detailed study of the auditory function of these patients has been reported[84].

Hearing and balance impairment caused by hair cell loss or dysfunction is a high-prevalence multifactorial disease that currently has no restorative treatment available. IGF-I and insulin, alone or in combination with other growth and neurotrophic factors, protect otic cells from ototoxic damage and promote *in vitro* hair cell regeneration[141-149]. The potential of IGF-I in the treatment of neurodegenerative diseases, together with the reported actions of IGF-I in inner ear development and function, support the hypothesis that this factor is a good candidate for inner ear regeneration therapy.

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