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# Growth hormone in the eye: A comparative update

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ABSTRACT

Comparative studies have previously established that the eye is an extrapituitary site of growth hormone (GH) production and action in fish, amphibia, birds and mammals. In this review more recent literature and original data in this field are considered.



## 1. Introduction

It is now well established that the eye is an extrapituitary site of growth hormone (GH) gene expression (Harvey et al., 2007a). Indeed, the presence of GH in the visual systems of fish, amphibia, birds and mammals has been described and putative roles for GH in vision and visual disease have been proposed (Harvey et al., 2007a,b). More recent studies that extend the literature in this field and additional original data are considered in the current review.

## 2. GH in reptilian eyes

GH has now been reported to be expressed in the retina of the green iguana (Ávila-Mendoza et al., 2015) as shown in Fig. 1. A Western blot of proteins extracted from the eyes of adult iguanas shows that, under reducing conditions, an immunoreactive GH moiety of 26 kDa is clearly present in the iguana pituitary gland (giPit) similar to that seen in the positive control, reflecting monomer recombinant derived chick GH (rcGH) (Fig. 1). In addition, an abundant smaller submonomeric GH variant (15 kDa) is also present, which resembles an analogous GH form that has been described in the chicken cerebellum (Alba-Betancourt et al., 2011; Arámburo et al., 2014) and retina (Baudet et al., 2007a). RT-PCR of mRNA extracted from the iguana neural retina shows that it can express the GH gene, as shown by the positive control, extracted from iguana hypothalamic tissue (Fig. 2). The iguana neural retina is also a site of GH action, as RT-PCR also shows that it expresses the GH receptor (GHR) gene, as also seen in hypothalamic extracts (Fig. 2). The iguana neural retina also contains transcripts for insulin-like growth factor-I (IGF-I) and for the neuropeptides GH-releasing hormone (GHRH), pituitary adenylate-cyclase activating peptide (PACAP), thyrotropin releasing hormone (TRH) and somatostatin (SRIF), as similarly found in extracts of the iguana hypothalamus (see Table 1). These neuropeptides may be involved in the local regulation of retinal GH in the iguana, as in chick eyes (Harvey et al., 2012). Immunohistochemistry also showed the presence of GH-immunoreactivity in the iguana neural retina (Fig. 3), particularly in retinal ganglion cells (RGCs) in the ganglion cell layer (GCL) and in small cells within the inner nuclear layer (INL). Retinal GH is therefore present in reptile eyes, as in other vertebrates.

## 3. GH in avian eyes

Since our review in 2007 (Harvey et al., 2007a,b), the nucleotide sequence of the small chicken GH (scGH) variant was reported and its expression in the retina detailed (Baudet et al., 2007a). The translated protein was determined using a specific antibody against the unique N-terminus of scGH and immunoreactivity was associated with a protein of 16 kDa, comparable with its predicted size. Most of the scGH immunoreactivity was, however, associated with a 31-kDa moiety, suggesting scGH is normally present in a dimerized form. Neither protein was, however, present in media of HEK cells, which had been transfected, with scGH DNA

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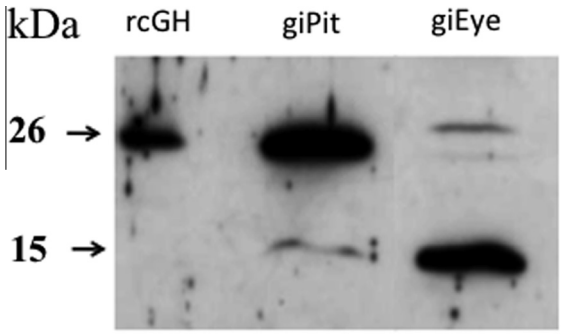


**Fig. 1.** Identification of growth hormone in the green iguana eye. GH immunoreactive bands were identified in iguana eye by SDS–PAGE under reducing conditions and Western-blot using a heterologous antibody directed against chicken GH (1:10,000) as previously described by Ávila-Mendoza et al. (2014). Recombinant cGH (rcGH) and green iguana pituitary extract (giPit) were used as positive control. Arrows indicate the approximate molecular weight and indicate that, in the eye, the most abundant immunoreactive band is a fragment of about 15 kDa.

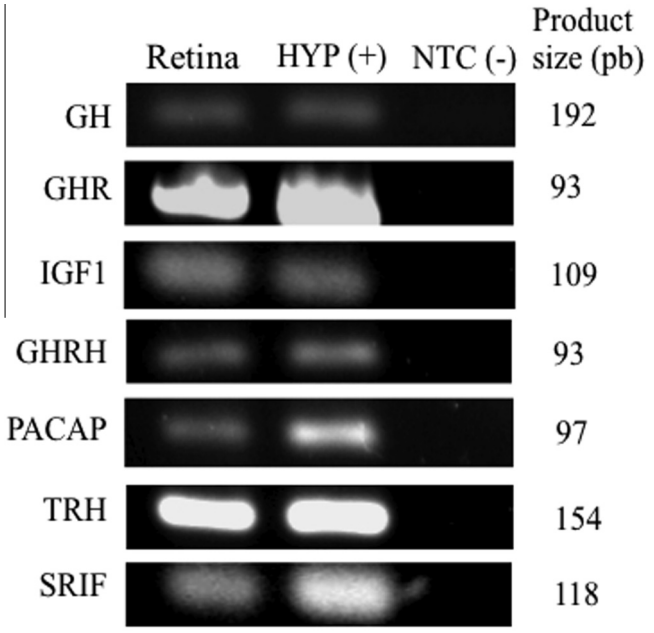


**Fig. 2.** Expression of GH, GHR, IGF-1, GHRH, PACAP, TRH and SRIF cDNAs in green iguana retina. Total RNA was extracted from 100 mg of hypothalamic or retinal tissues using 1 ml of *TRIzol Reagent* (Invitrogen, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA contamination was removed by incubating RNA with 1 U of *DNase I* (Invitrogen) at 37 °C for 15 min, followed by enzymatic inactivation at 70 °C for 15 min. First-strand cDNAs, were synthesized from 2 μg total RNA using 100 U of *Superscript II* reverse transcriptase (Invitrogen), 0.5 μg oligo d(T), 0.5 μg random hexamers and 1 mM dNTPs for 50 min at 42 °C followed by 15 min at 70 °C. Each reaction included 2 μL cDNAs, 0.5 μM of each specific primer, dNTPs mix 0.5 mM (Invitrogen), $MgCl_2$ 1.5 mM, and 2.5 U of *Taq* polymerase (Invitrogen) in a 25 μL final volume. PCR cycling was performed as follows: 95 °C for 5 min, 72 °C for 3 min and followed by 35 cycles of 95 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min. Amplification products were resolved 1% agarose Tris–Acetate–EDTA gels stained with BrEt and observed in the *GEL DOC EZ Imager* (BioRad, Hercules, CA, USA). With the synthesized cDNA, GH, GHR, IGF-1, GHRH, PACAP, TRH and SRIF cDNAs were amplified using specific primers (Table 1). As negative control (NTC), reactions were carried out in absence of DNA template. (bp) base pairs corresponding to band size.

after its insertion into an expression plasmid. This suggests scGH is not a secretory product, consistent with its lack of a signal peptide sequence. Similar scGH moieties of 16 and 31 kDa were also found in proteins extracted from ocular tissues (the neural retina, pigmented epithelium, cornea and choroid) of chick embryos,

**Table 1**
Oligonucleotide primer sequences.

| Name | Target | Primer | Sequence (5′–3′) |
|---|---|---|---|
| giGHRH-qf | GHRH | Forward | CTGTCAGCCCAGAAGTTATTGC |
| giGHRH-qr | GHRH | Reverse | TCGCCTGGTCAAGAATTCGT |
| giPACAP-qf | PACAP | Forward | ACACCTTGTATTATCCGCCAGA |
| giPACAP-qr | PACAP | Reverse | AGATACTTCCTCGCAGACAGC |
| giTRH-qf | TRH | Forward | CACCCTGGCAAACGAAGCTG |
| giTRH-qr | TRH | Reverse | TGCCTTTGCTGCATTCCAA |
| giSST-qf | SRIF | Forward | AACCCGGCGATTTGTCCC |
| giSST-qr | SRIF | Reverse | AAGTTCTTGCATCCCGCTTT |
| giGH-qf | GH | Forward | AGAAGTTTGAATCCAACCTCCG |
| giGH-qr | GH | Reverse | AGATATGTCTCCACCTTGTGC |
| giGHR-qf | GHR | Forward | CCTCCTGTTCCAGTACCCAA |
| giGHR-qr | GHR | Reverse | AGTATCTGAGCCTTCGCTCC |
| giIGF1-qf | IGF-1 | Forward | GCAACATTCATCGACTATGCCT |
| giIGF1-qr | IGF-1 | Reverse | CGCCCTTCAGTTTGTCTGC |

although they were not consistently present in the vitreous humor, again indicating its lack of secretion within the eye. Specific scGH immunoreactivity was also detected in chick ocular tissues by immunohistochemistry, but not in axons of the optic fiber layer (OFL) or the optic nerve head (ONH), which were both immunoreactive for full-length chicken GH. Thus although scGH is expressed and translated in chick ocular tissues, its localization in the neural retina and ONH is distinct from that of the full-length protein. Structure–function relationships of this variant were subsequently assessed (Baudet and Harvey, 2007), as it lacks many of the conformational sites required for its interaction with the classical GH receptor (GHR) and it is unlikely to be involved in GH signaling (Harvey et al., 2014). The secondary structure of the C-terminus of scGH is similar to the C-terminus of hGH (which can bind to the chick GHR), but the N-terminus is severely truncated, lacking residues derived from intron C of the full-length cGH. Indeed, the predicted structure of its N-terminus has no classical secondary structure (alpha-helix or beta sheet), whereas the N-terminus of hGH is composed of helix 1 and two mini-helices located between helix 1 and helix 2. This difference in ribbon structure results in a difference in the overall shape of scGH that precludes its activation of the GHR. hGH, in contrast, binds to the extracellular domain (ECD) of two GHRs sequentially at its binding site 1 (high affinity site) and then at its binding site 2 (or low affinity site). Sequence alignment of scGH with hGH showed that scGH lack three key residues (of 14) at site 1 and nine residues (of 15) at site 2. It is therefore unlikely that tight binding of ECD1 to site 1 of scGH could occur. scGH also lacks most of the site 2 residues, suggesting that it is unlikely that ECD2 would induce GHR signaling. It is therefore curious and enigmatic that the immunoneutralization of endogenous GHR was accompanied by increased RGC death (Baudet et al., 2007a), suggesting a physiological role for scGH in RGC survival. However, while some biological effects of monomer GH or its proteolytic fragments have been described (Alba-Betancourt et al., 2013; Harvey et al., 2014), scGH is unlikely to act via classical GHRs in ocular tissues (Harvey et al., 2014).

The presence of GH and its receptor in chick embryo RGC axons that project to and innervate visual centers of the brain were mapped throughout the last trimester of the 21 days incubation (Baudet et al., 2007b). Immunoreactivity for GH was demonstrated in fascicles of the OFL, formed by axons underlying the RGCs. Immunoreactivity was also traced through the optic nerve through the optic chiasm, into the optic tract and into the stratium opticum and into retinorecepient layers of the optic tectum, where the RGCs axons synapse. The presence of GH immunoreactivity in the tectum occurs prior to synaptogenesis with the RGC axons and thus reflects the local expression of the GH gene, especially as GH mRNA was also distributed within this tissue. The distribution of the