Growth hormone-releasing hormone receptor signaling in experimental ocular inflammation and neuroprotection

https://doi.org/10.4103/1673-5374.336135

Date of submission: September 13, 2021

Date of decision: November 29, 2021

Date of acceptance: December 15, 2021

Date of web publication: April 29, 2022

From the Contents

Ling-Ping Cen[1, #], Tsz Kin Ng[1, 2, 3, #], Wai Kit Chu[3], Chi Pui Pang[1, 3, *]

Abstract

Both inflammation and anti-inflammation are involved in the protection of retinal cells. Antagonists of the hypothalamic growth hormone-releasing hormone receptor (GHRHR) have been shown to possess potent anti-inflammatory properties in experimental disease models of various organs, some with systemic complications. Such effects are also found in ocular inflammatory and neurologic injury studies. In experimental models of mice and rats, both growth hormone-releasing hormone receptor agonists and antagonists may alleviate death of ocular neural cells under certain experimental conditions. This review explores the properties of growth hormone-releasing hormone receptor agonists and antagonists that lead to its protection against inflammatory responses induced by extrinsic agents or neurologic injures in ocular animal models.

Key Words: agonists; antagonists; growth hormone-releasing hormone receptor; macrophages; neuroprotection; ocular inflammation; optic nerve injury; retinal ganglion cells; uveitis

Introduction

Both inflammation and anti-inflammation paradoxically play roles in neuroprotection of the retina. The underlying mechanisms are complex and controversial. We have demonstrated that mild inflammation could promote retinal ganglion cell (RGC) survival and axonal regeneration in rats after optic nerve (ON) injury (Cen et al., 2018; Liu et al., 2021). In contrast, anti-inflammatory effects are also involved in protecting RGC from the effects of injury in rats (Yang et al., 2019), as well as preserving retinal functions in an experimental autoimmune uveoretinitis model (Li et al., 2019). Among the intrinsic mechanisms associated with anti-inflammation, hypothalamic growth hormone-releasing hormone (GHRH) exerts extra-pituitary functions in mediating serial inflammatory processes in different organ systems and diseases (Barabutis and Schally, 2010). GHRH is a hypothalamic releasing hormone with a physiological role in regulating the synthesis and release of hormones in the anterior pituitary gland (Schally and Bowers, 1964). Hypothalamic hormones including luteinizing hormone-releasing hormone (LHRH), GHRH, thyrotropin releasing hormone (TRH), gastrin releasing peptide (GRP) and somatostatin as well as their respective receptors, including the LHRH receptor, GHRH receptor (GHRHR), TRH receptor, GRP receptor and somatostatin receptor-1 have been well studied. Notably, they are expressed in many eye tissues: conjunctiva, cornea, trabecular meshwork, ciliary body, lens, retina, and ON (Dubovy et al., 2017). The GHRHR signaling pathway of the GHRH-GH-insulin like growth factor 1 axis could be involved in the regulation of ocular physiological and pathological conditions. Since then, vigorous research programs have been conducted to investigate GHRHR agonists and antagonists. This article reviews the properties of synthetic GHRH analogs and their actions on GHRHR. In addition, recent findings of GHRH on neuroprotection in experimental ON injury and ocular inflammation are also summarized.

Search Strategy and Selection Criteria

Bibliographic citation database searched: PubMed. Selection criteria: the articles were searched with keywords "GHRH" in combination of other specific terms/diseases. Retrieval time: From July 13 to November 27, 2021. The inclusion of the articles was determined by their experimental results.

Growth Hormone-Releasing Hormone and Its Receptor

Investigations of the GHRH-GHRHR-GH axis

The human GHRH gene, located on chromosome 20q11.23, codes for a neurohumoral polypeptide which belongs to the glucagon family of proteins. Its preproprotein is produced and released by GHRH-producing neurons in the hypothalamus (Bloch et al., 1983). It is cleaved to generate the mature somatoliberin, which binds to its receptor GHRHR (**Figure 1**) in the anterior pituitary gland (Mayo, 1992). GHRHR stimulates the synthesis and release of growth hormone (GH; Schally et al., 1968) which, after release from the anterior pituitary, circulates in the bloodstream and stimulates the synthesis and secretion of insulin-like growth factor-I (IGF1) in the liver (Schwander et al., 1983). This is the canonical hypothalamic GHRH-pituitary GH-liver IGF1 axis (Bernasconi et al., 1999). GH concentration in the body, regulated by both GHRH and somatostatin, is important for gains of body size and weight. The biogenesis of GHRH has been persistently explored in bioengineered rodents. In *Ghrh*$^{-/-}$ mice with CRISPR/Cas9-mediated loss-of-function in the *Ghrh* gene, there were significant reductions of body weight, lean mass, bone mineral contents and bone density but increases in fat mass with enhanced insulin sensitivity as compared to wildtype mice (Icyuz et al., 2020). They also showed dramatic reductions in oxygen consumption, carbon dioxide production, energy expenditure, and respiratory exchange ratio during the light cycle. Moreover, mice with homozygous ablation of the *Ghrh* gene showed weakened performance in learning and memory tests and age-related decline in cognitive functions, but increase in spontaneous locomotor activity (Leone et al., 2018). Interestingly, *Ghrh*-knockout (KO) mice are remarkably long-lived with increased adiponectin levels and alterations in glucose homeostasis, with enhanced expressions of genes related to xenobiotic detoxification, stress resistance, and insulin signaling (Sun et al., 2013). The lifespan of *Ghrh*-KO mutants could be further increased with caloric restriction.

[1]Joint Shantou International Eye Center of Shantou University and The Chinese University of Hong Kong, Shantou, Guangdong Province, China; [2]Shantou University Medical College, Shantou, Guangdong Province, China; [3]Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China

*Correspondence to: Chi Pui Pang, DPhil, cppang@cuhk.edu.hk.

http://orcid.org/0000-0003-3876-0606 (Chi Pui Pang)

#These authors contributed equally to this work.

Funding: *This work was supported by the National Natural Science Foundation of China (81570849 to LPC), Joint Regional Basic Science and Applied Basic Science Research Fund of Guangdong Province (2019A1515110685 to TKN), Special Fund for Chinese Medicine Development of Guangdong Province (20202089 to TKN), the Natural Science Foundation of Guangdong Province (2020A1515010415 to LPC), an internal grant from Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong, Grant for Key Disciplinary Project of Clinical Medicine under the Guangdong High-level University Development Program, China, and The Chinese University of Hong Kong Direct Grant (2020.067 to WKC).*

How to cite this article: *Cen LP, Ng TK, Chu WK, Pang CP (2022) Growth hormone-releasing hormone receptor signaling in experimental ocular inflammation and neuroprotection. Neural Regen Res 17(12):2643-2648.*

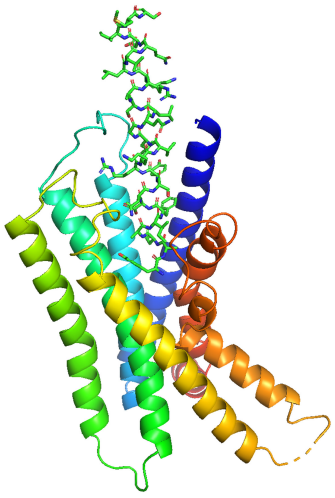

Figure 1 | Protein structure of human growth hormone-releasing hormone binding to growth hormone-releasing hormone receptor.
Schrödinger docking simulation of growth hormone-releasing hormone binding to AlphaFold-predicted three-dimensional structure of human growth hormone-releasing hormone receptor protein (Tunyasuvunakool et al., 2021).

Microsatellite markers D20S45 and D20S54, located approximately 1 and 3 cM from GHRH respectively, have been linked with isolated GH deficiency (Pérez Jurado et al., 1994). But so far no disease causing mutation has been identified in the human *GHRH* gene (Mullis, 2011). Instead, loss-of-function mutations in the *GHRHR* gene, located on chromosome 7p14.3, were found in patients with familial GH deficiency who presented with extremely short stature, no dysmorphy, and unresponsive to exogenous GHRH, but with prolactin levels within the lower normal range (Netchine et al., 1998). Over 50 human *GHRHR* sequence variants in association with growth complications have been identified including truncation and missense mutations; GHRHR p.Glu72 is a common mutation in GH deficiency (Cohen et al., 2019; Kale et al., 2020) that leads to dwarfism in human. GHRHR, belonging to the family of G protein-coupled receptors with seven transmembrane domains which activate adenylyl cyclase, is expressed on pituitary somatotropic cells and is a mediator of the GHRH-GH-IGF1 axis. Experimental autoimmune encephalomyelitis (EAE) was alleviated in both *Ghrh*-KO and *Ghrhr*-KO mice (Shohreh et al., 2011). Notably, GH supplementation was able to restore the EAE susceptibility in the *Ghrh*-KO mice, suggesting GH, but not GHRH, is involved in the development of EAE (Shohreh et al., 2011). Downstream of the GHRH-GHRHR-GH axis, IGF1 has also been reported to affect the development of EAE in mice (DiToro et al., 2020). Mechanically, IGF1 receptor could activate the AKT-mTOR pathway and increase aerobic glycolysis, which could enhance T helper 17 cell differentiation more than that of regulatory T cells and enhance expressions of pro-inflammatory genes that lead to EAE (DiToro et al., 2020). Apart from the recognized regulatory function of GHRH-GHRHR-GH in the brain, the presence of a spermatogenic-specific promoter in the rat GHRH gene (Srivastava et al., 1995) shows that the GHRH-GH axis has functions outside the hypothalamus and pituitary. The expressions of GHRH and GHRHR in different organs and tissues by RNA-sequencing analysis (**Table 1**) suggests non-canonical GHRH-GH functions.

Chemical structures and biochemical properties of GHRHR agonists and antagonists

GHRH, a neurosecretory peptide synthesized in the hypothalamus, is chemically an acidic polypeptide that can be isolated from the acetic acid extracts of hypothalamic tissues (Schally et al., 1969). GHRH isolated from various mammalian species including rodents and primates shares similar chemical characteristics and cross-species biological activities (Schally et al., 1970). Fragments containing the first 29 amino acids of human GHRH could be chemically synthesized by a solid-phase peptide synthesis technique, and demonstrated high biological activity (Felix et al., 1988). Subsequently, different modified analogs of human GHRH have also been generated, including replacement of the 29th amino acid residue of arginine by agmatine, which was obtained by decarboxylation of arginine (Kovacs et al., 1988). Compared to human GHRH-(1-29)-NH_2, subcutaneous administration of these synthetic modified GHRH agonists could be 45–96 times more potent in stimulation of GH release, at 15 minutes and 88–220 times more active at 30 minutes with higher binding affinities for GHRH receptors on rat pituitary cells (Izdebski et al., 1995). C-terminal methylamide and ethylamide analogs of human GHRH-(1-29)-NH_2, such as MR-403 (N-Me-Tyr^1 D-Ala^2 Arg^{29}-$NHCH_3$-JI-38), MR-406 (N-Me-Tyr^1 Arg^{29}-$NHCH_3$-JI-38), MR-409 (N-Me-Tyr^1 D-Ala^2 Asn^8 Arg^{29}-$NHCH_3$-JI-38) and MR-410 (N-Me-Tyr^1 D-Ala^2 Thr^8 Arg^{29}-$NHCH_3$-JI-38), showed dramatic increase in endocrine activities (100–220 times more potent at 15 minutes and 360–450 times more active at 30 minutes) as compared to human GHRH-(1-29)-NH_2. They are among the most potent GHRH agonistic analogs that have been chemically synthesized (Cai et al., 2014).

In the course of synthesis of various analogs of hGHRH-(1-29)NH_2, it was found that the replacement of Ala^2 by D-Arg^2 led to antagonists (Robberecht et al., 1985). The GHRH antagonist [Ac-Tyr^1 D-Arg^2] human GHRH-(1-29)NH_2 blocked endogenous and GHRH-stimulated GH secretion after injection to rats (Waelbroeck et al., 1985). Since D-Arginine at position 2 appeared to be essential for GHRH antagonistic activity, all GHRH antagonists contained D-Arginine at amino acid position 2 (Sato et al., 1987). Some amino acid substitutions in human GHRH-(1-29)NH_2 could produce more potent antagonists (Coy et al., 1991) and increase affinity to GHRHR (Goudreau et al., 1992). Even more potent antagonists were produced by further modifying these analogs with non-coding amino acids as follows: D-Arg^2, Phe(4-Cl)^6 (para-chlorophenylalanine), Abu^{15} (alpha-aminobutyric acid), Nle^{27} and Agm^{29} (agmatine). Antagonists MZ-4-71 [Ibu^0 D-Arg^2 Phe(4-Cl)^6 Abu^{15} Nle^{27}]-human GHRH-(1-28)Agm, MZ-4-169 [Nac^0 D-Arg^2 Phe(4-Cl)^6 Abu^{15} Nle^{27}]-human GHRH-(1-29) NH_2, and MZ-4-181 [Nac^0-His^1 D-Arg^2 Phe(4-Cl)^6 Abu^{15} Nle^{27}]-human GHRH-(1-29)NH_2 induced significantly greater inhibition of GH release than did the standard antagonist [Ac-Tyr^1 D-Arg^2]-human GHRH-(1-29)NH_2 (Zarandi et al., 1994). Other potent GHRH antagonists have also been reported: MIA-313 ([(Ac-Amc)^0-Tyr^1 D-Arg^2 Cpa^6 Ala^8 Har^9 Tyr(Me)^{10} His^{11} Orn^{12} Abu^{15} His^{20} Nle^{27} D-Arg^{28} Har^{29} Agm^{30}]-human GHRH(1-30), MIA-602 ([(PhAc-Ada)^0-Tyr^1 D-Arg^2 (Phe[F]5)^6 Ala^8 Har^9 Tyr(Me)^{10} His^{11} Orn^{12} Abu^{15} His^{20} Orn^{21} Nle^{27} D-Arg^{28} Har^{29}]-human GHRH(1-29)NH_2), MIA-604 ([(PhAc-Ada)^0-Tyr^1 D-Arg^2 (Phe[F]5)^6 Ala^8 Har^9 Tyr(Me)^{10} His^{11} Orn^{12} Abu^{15} His^{20} Orn^{21} Nle^{27} D-Arg^{28} Har^{29} Agm^{30}]-human GHRH(1-30), and MIA-610 ([PhAc^0-Tyr^1 D-Arg^2 Cpa^6 Ala^8 Har^9 (Phe[F]5)^{10} His^{11} Orn^{12} Abu15 His^{20} Orn^{21} Nle^{27} D-Arg^{28} Har^{29} Ada^{30}]-human GHRH(1-30) (Klukovits et al., 2012).

Physiological properties and clinical effects of GHRHR agonists and antagonists

In human, the main function of GHRH is regulation of the secretion of GH from the pituitary gland. But GHRH also possesses a wide range of extra-pituitary or peripheral activities in organs including brain, heart, kidney, and retina. On the cellular level, it enhances cell migration, proliferation, and differentiation. Specifically, it regulates proliferation and survival of pancreatic islet and β-cell cells, fibroblasts and endometrial cells and protects cardiomyocytes from apoptosis. Accordingly, GHRH promotes cardioprotection, enhances wound healing, affects the immune systems and reduces inflammation (Kriaris et al., 2011; Cai et al., 2014). Binding assays confirmed that the first 29 amino acids of the 44-residue human GHRH is critical for its full biological activities, especially in GHRHR activation, GHRHR affinity and peptide stability (Gaudreau et al., 1992).

Clinical effects of GHRH have long been known. Subcutaneous infusion of GHRH has been shown to restore GH secretion, promote nitrogen retention and raise the level of serum somatomedin C and accelerate linear growth in children with GH deficiency (Thorner et al., 1985). However, large individual variations in responses to GHRH. Some GH-deficient children (Biscaldi et al., 1990) and obese patients (Kopelman et al., 1985) do not respond to GHRH treatment. For example, GHRHR antagonist MIA-690, rather than GHRHR agonist MR-409, increased food intake and body weight in mice. MIA-690 neither changed locomotor activity nor alterations in subcutaneous, visceral or brown adipose tissue masses. But expression of the hypothalamic agouti-related peptide gene and norepinephrine levels were increased, and serotonin was reduced (Recinella et al., 2021). These indicate the presence of complex pulsatile regulation of GH secretion and somatic growth with involvements of extra-pituitary functions of GHRH. More intensive studies on the basic biochemical and physiological properties of GHRH and its agonists and antagonists are warranted.

Studies of Growth Hormone-Releasing Hormone Receptor Agonists and Antagonists in Disease Models

Heart diseases

The peptide GHRH(1-44)NH_2 attenuated phenylephrine-induced hypertrophy in H9c2 cardiac cells, adult rat ventricular myocytes and human induced pluripotent stem cell-derived cardiomyocytes *in vitro* by blockade of phospholipase Cβ, protein kinase Cε, calcineurin, phospholamban and Gq signaling (Gesmundo et al., 2017). GHRH-agonist JI-38 reversed ventricular remodeling and enhanced functional recovery in rats with chronic myocardial infarction by improving cardiac function, reducing myocardial infarction size and increasing myocyte and nonmyocyte mitosis without increases in circulating GH or IGF1 (Kanashiro-Takeuchi et al., 2012). Moreover, by activating the ERK and AKT pathways, GHRH agonist JI-38 could increase the division of porcine cardiac stem cells and reduce cell death upon exposure to hydrogen peroxide (Florea et al., 2014). The GHRH agonist MR-409 could mitigate cardiac hypertrophy in mice with transverse aortic constriction and enhance the cardiac function by improving cardiomyocyte contractility and altering the sarcolemmal structure (Gesmundo et al., 2017). In a recent study, treatment of mice by GHRH agonist MR-409 for 6 months reversed aging-associated changes in heart function, mobility, hair growth, cellular energy production and cell senescence by improving mitochondrial functions and reducing cardiac inflammation (Xiang et al., 2021).

Cancer

GHRHR was highly expressed in human retinoblastoma cells and gastric cancer tissue cells (Chu et al., 2016; Gan et al., 2016). Two different GHRHR antagonists, MIA-602 and MIA-690, significantly enhanced apoptosis in retinoblastoma cells, likely via the upregulation of caspase-3 and downregulation of the extracellular regulated protein kinases (ERK) 1/2 pathways (**Table 2**). Retinal pigment epithelial cells had no response towards these two GHRHR antagonists. Targeting GHRHR therefore may induce apoptotic cell death in retinoblastomas without severe harm to other retinal cells. Tumorigenesis involves inflammation and its key signaling molecules, nuclear factor kappa-B (NF-κB) and signal transducer and activator of transcription 3 (STAT3; Shrihari, 2017). We have identified GHRHR as an

Table 1 | **RNA-sequencing expression of GHRH, GHRHR, GH, GHR and IGF1 in normal tissues**

Tissues	GHRH RPKM (count)	GHRHR RPKM (count)	GH1 RPKM (count)	GHR RPKM (count)	IGF1 RPKM (count)
Adrenal	0.054±0.076 (330)	0.178±0.041 (3156)	0.005±0.005 (40)	2.370±0.144 (162800)	0.480±0.134 (68324)
Appendix	0.000±0.000 (0)	0.000±0.000 (0)	0.021±0.022 (202)	0.779±0.082 (47914)	3.007±0.756 (367080)
Bone marrow	0.000±0.000 (0)	0.000±0.000 (0)	0.083±0.036 (2087)	0.023±0.011 (4144)	0.027±0.025 (9523)
Brain	0.073±0.062 (560)	0.000±0.000 (0)	0.021±0.029 (233)	0.645±0.077 (51649)	0.240±0.054 (40056)
Colon	0.007±0.013 (117)	0.015±0.016 (420)	0.000±0.000 (0)	2.230±0.647 (398951)	0.650±0.171 (242822)
Duodenum	0.910±0.108 (3395)	0.017±0.017 (197)	0.088±0.088 (544)	0.444±0.092 (18936)	0.376±0.002 (32257)
Endometrium	0.000±0.000 (0)	0.003±0.004 (38)	0.145±0.103 (1142)	1.598±0.219 (118019)	6.958±6.251 (1463639)
Esophagus	0.020±0.028 (183)	0.000±0.000 (0)	0.025±0.018 (388)	1.694±0.224 (192102)	0.753±0.305 (176000)
Fat	0.000±0.000 (0)	0.040±0.029 (837)	0.000±0.000 (0)	43.394±4.104 (3058842)	6.281±0.193 (899883)
Gall bladder	0.000±0.000 (0)	0.123±0.087 (3848)	0.000±0.000 (0)	2.513±0.343 (282990)	2.544±0.713 (576743)
Heart	0.509±0.698 (7204)	0.000±0.000 (0)	0.014±0.024 (328)	2.496±0.523 (398025)	0.856±0.716 (288044)
Kidney	0.000±0.000 (0)	0.043±0.028 (872)	0.000±0.000 (0)	4.394±0.872 (376728)	0.163±0.028 (27178)
Liver	0.000±0.000 (0)	0.000±0.000 (0)	0.000±0.000 (0)	35.041±12.088 (3264710)	3.510±2.423 (450053)
Lung	0.000±0.000 (0)	0.005±0.009 (61)	0.037±0.064 (730)	1.794±0.424 (223052)	0.434±0.170 (119929)
Lymph node	0.000±0.000 (0)	0.002±0.003 (101)	0.106±0.087 (2720)	0.534±0.245 (104842)	0.594±0.190 (236737)
Ovary	0.000±0.000 (0)	0.000±0.000 (0)	0.000±0.000 (0)	3.294±0.680 (295055)	0.730±0.466 (136007)
Pancreas	0.000±0.000 (0)	0.009±0.009 (236)	0.000±0.000 (0)	0.185±0.049 (16907)	0.057±0.008 (10125)
Placenta	0.000±0.000 (0)	0.000±0.000 (0)	12.559±18.947 (252319)	1.780±0.506 (278108)	2.676±1.245 (841875)
Prostate	0.000±0.000 (0)	0.022±0.014 (548)	0.019±0.032 (202)	4.886±0.831 (439662)	3.522±0.877 (634213)
Salivary gland	0.000±0.000 (0)	0.004±0.005 (133)	0.005±0.006 (103)	0.519±0.053 (75803)	0.238±0.069 (68367)
Skin	0.000±0.000 (0)	0.000±0.000 (0)	0.029±0.041 (505)	1.623±0.698 (198114)	0.023±0.006 (5824)
Small intestine	0.404±0.389 (3288)	0.003±0.006 (87)	0.018±0.031 (279)	0.865±0.279 (87522)	0.568±0.183 (118094)
Spleen	0.000±0.000 (0)	0.000±0.000 (0)	0.237±0.116 (4272)	1.460±0.323 (190009)	0.943±0.163 (247363)
Stomach	0.273±0.386 (1944)	0.000±0.000 (0)	0.039±0.033 (493)	0.902±0.563 (79304)	0.318±0.194 (56512)
Testis	0.015±0.024 (382)	0.016±0.015 (1191)	0.044±0.019 (1758)	1.039±0.506 (296126)	1.149±0.483 (672816)
Thyroid	0.000±0.000 (0)	0.002±0.004 (101)	0.020±0.013 (524)	1.659±0.478 (266627)	0.393±0.402 (126746)
Urinary bladder	0.000±0.000 (0)	0.000±0.000 (0)	0.021±0.021 (224)	1.471±0.639 (107722)	2.398±0.904 (358421)

Data in Table 1 were obrained from Human Protein Atlas RNA-seq normal tissues project on 27 different tissues from 95 human individuals (Fagerberg et al., 2014). GH: Growth hormone; GHRH: growth hormone-releasing hormone; GHRHR: growth hormone-releasing hormone receptor; IGF1: insulin-like growth factor-I; RPKM: reads per kilo base per million mapped reads.

Table 2 | **Summary of GHRHR agonists and antagonists on ocular diseases**

GHRH analogs	Ocular disease model	Effect	References
GHRHR agonist MR-409	Streptozotocin-induced diabetic rats	• Prevents retinal morphological alteration induced by hyperglycemia, preserving RGC survival. • Upregulates NRF-2-dependent gene expression • Downregulates proinflammatory cytokines and adhesion molecules. • Downregulates VEGF expression while increases pigment epithelium-derived factor expression.	Thounaojam et al., 2017
	Optic nerve injury rat model	• Subcutaneous application promotes the survival of RGCs. • Further enhances the promotion of RGC survival by lens injury or zymosan-induced macrophage activation.	Cen et al., 2021
GHRHR antagonist MIA-602	Endotoxin-induced uveitis rat model	• Decreases LPS-stimulated surges of GH and IGF1 in aqueous humor. • Reduces infiltration of macrophages and leukocytes and production of TNF-α, IL-1β and MCP-1.	Qin et al., 2014
		• Suppresses phosphorylation of STAT3. • Attenuates expression of downstream proinflammatory factors after LPS treatment.	Liang et al., 2020
	LPS-treated rat ciliary body and iris explant culture	• Suppresses the elevated expression of IL-1β and IL-6 and reduces the release of IL-6.	Ren et al., 2019
	Retinoblastoma cell line	• Induces retinoblastoma cell apoptosis. • Downregulates cell proliferation genes and upregulates apoptotic genes.	Chu et al., 2016
	Primary pterygium epithelial cell culture	• Induced apoptosis of pterygium epithelial cell s in a dose-dependent manner. • Downregulates ERK1 and upregulates caspase-3 expression.	Qin et al., 2018
	Optic nerve injury rat model	• Subcutaneous application promotes the survival of RGCs	Cen et al., 2021
GHRHR antagonist MIA-690	Retinoblastoma cell line	• Induces retinoblastoma cell apoptosis. • Downregulates cell proliferation genes and upregulates apoptotic genes.	Chu et al., 2016

ERK1: Extracellular regulated protein kinases 1; GH: growth hormone; GHRH: growth hormone-releasing hormone; IGF1: insulin-like growth factor-I; IL-1β: interleukin-1β; IL-6: interleukin-6; LPS: lipopolysaccharide; NRF-2: nuclear factor erythroid 2-related factor 2; MCP-1: monocyte chemotactic protein-1; PEDF: pigment epithelium-derived factor; RGC: retinal ganglion cells; STAT3: signal transducer and activator of transcription 3; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

important mediator in experimental ocular inflammation (Qin et al., 2014; Ren et al., 2019; Liang et al., 2020). By applying the GHRHR antagonist MIA-602, multiple human gastric cancer cell lines showed slower proliferation both *in vitro* and in mouse xenografts. Mechanistically, MIA-602 could suppress the phosphorylation of STAT3 and the expression of the p65 subunit of NF-κB, as well as their upstream activator PAK1. The GHRHR mediated PAK1-STAT3/NF-κB pathway should be explored for its potential as a novel avenue for gastric cancer treatment.

The GHRHR splicing variant SV1, but not full length GHRHR, was detected in human esophageal squamous cell carcinoma (ESCC) cells where its expression was significantly higher than in adjacent non-cancerous tissues and was positively correlated with the tumor dimension, pathological nodal status, pathological stage of tumour nodal metastasis, and overall survival in ESCC patients (Xiong et al., 2020). SV1 mediated GHRHR antagonist inhibitory effects in the absence of the full length GHRHR. The GHRHR antagonist MIA-602 was able to inhibit ESCC cell viability, migration and invasion. Upon the overexpression of splice variant 1 (SV1), NF-κB was upregulated, which in turn enhanced the expression of glycolytic enzyme human phosphofructokinase, muscle (PFKM). These results demonstrated that MIA-602 inhibited ESCC growth through the SV1-NF-κB-PFKM signaling pathway.

Alzheimer's disease

The reduction of somatostatin concentration in several brain areas of patients with Alzheimer's disease was shown to be associated with delayed GH response to GHRH in these patients (Nemeroff et al., 1989). In a clinical trial daily treatment with GHRH ameliorated age-related cognitive declines in healthy elderly (Vitiello et al., 2005). Similarly, a randomized, double-blind and placebo-controlled trial study reported that twenty weeks of daily subcutaneous injections of a stabilized analog of human GHRH (tesamorelin; Theratechnologies Inc., Montréal, Canada) showed enhancing effects on cognition and executive functions in both healthy older adults and adults with mild cognitive impairment (Baker et al., 2012). Increased levels of glutamate

and the inhibitory transmitter γ-aminobutyric acid were detected in all 3 brain regions examined (dorsolateral frontal, posterior cingulate, and posterior parietal), while N-acetylaspartylglutamate was increased in the frontal cortex and myo-inositol was decreased in the posterior cingulate (Friedman et al., 2013). In another study, however, plasma neuronally-derived exosomes concentrations of ptau-S396 and growth-associated protein 43 were not affected by GHRH treatment (Winston et al., 2018).

Experimental studies have revealed more intriguing properties of GHRH. In early-onset Alzheimer's disease model APPswe/PS1ΔE9 transgenic mice, intraperitoneal injection of GHRH at dark onset increased sleep and decreased brain interstitial fluid amyloid-β. Delivery of a GHRH antagonist [Phenylacetyl-(D-Arg^{2}.28 p-chloro-Phe^{6} Homoarg^{9}.29 Tyr(Me)^{10} Abu^{15} Nle^{27})-GRF (1-29) NH_2] via reverse-microdialysis suppressed sleep and increased brain interstitial fluid amyloid-β (Liao et al. 2015). On the contrary, GHRH antagonist MZ-4-71 corrected the impaired memory consolidation caused by β-amyloid 25–35 in mice (Telegdy et al., 2011). GHRH antagonist MIA-690 decreased escape latency in genetically modified Alzheimer's disease model 5XFAD mice and reduced the concentrations of amyloid-β1–42 and τ filaments in brain samples by its anti-oxidative and neuro-protective properties (Jaszberenyi et al., 2012). Positive treatment effects of both GHRH agonist and antagonist indicate differential mechanisms of the treatment effects in different cell types by these GHRH analogs. However, it remains to be investigated whether there is an optimal GHRH activity level in an "U-shaped curve" in that both too little and too much GHRH activity can be.

Diabetic retinopathy

Diabetic retinopathy (DR), a common and serious complication of diabetes mellitus, is a leading cause of irreversible blindness. Based on the degree of neovascularisation, DR can be categorized into non-proliferative and proliferative DR. One key feature of proliferative DR is the formation of fibrovascular membranes (FVM) at the interface between the vitreous and the retina (Tamaki et al., 2016). In our recent study of proliferative DR, we detected significantly higher GHRH and GH levels in vitreous and aqueous collected from proliferative DR patients than those from non-diabetic patients (Qin et al., 2020). In FVM collected from proliferative DR patients, GHRH and GHRHR, but not IGF1, were detected in polymorphonuclear cells and vascular endothelial cells, suggesting involvement of GHRH signalling in the development of FVM in proliferative DR. Notably, GHRHR expression was reduced in retinas of diabetic patients and a diabetic rat model induced by streptozotocin (STZ) treatment (Thounaojam et al., 2017). In experimental diabetic retinopathy, there are retinal perturbations and inflammation, leading to consequential neuro-glial disruptions that are difficult to treat (Rübsam et al., 2018). In DR, the innate immune system involves complicated individual and interactive effects of competent cells, mediators and the complement system (Pan et al., 2021). Downstream of the GHRHR-GHRH-GH axis, IGF1 could protect RGCs from axon injury via the AKT pathway in rats (Rathnasamy et al., 2017). GHRHR agonist MR-409 was able to elevate GHRHR expression in STZ-rat retinas. More ganglion cell death was detected in diabetic than healthy rat retina, while this could be suppressed by MR-409 treatment (**Table 2**). Mechanistically, MR-409 was able to reduce oxidative stress and inflammation in diabetic rat retina. The lower oxidative stress caused by MR-409 treatment led to reduced retina levels of dihydroethidium, 3-nitrotyrosine and 4-hydroxynonenal. MR-409 also reduced expression of vascular endothelial growth factor and decreased retinal vascular permeability in diabetic rats. These experimental findings indicate potential for GHRHR agonists as a novel treatment for DR.

Growth Hormone-Releasing Hormone Receptor Agonist and Antagonist Treatment in Experimental Ocular Inflammation Models

Uveitis model

Uveitis is a sight-threatening disease, and about 35% of uveitis patients have visual impairment. The pathogenic basis of uveitis is extremely complex, with more than 60 etiologies known to account for uveal inflammation. Uveitis could be sub-classified into infectious and non-infectious types. Non-infectious uveitis is associated with systemic rheumatological and autoimmune diseases (Hsu et al., 2019). Among causes of non-infectious uveitis, Behcet's disease, sarcoidosis and Vogt-Koyanagi-Harada disease are more common in the Asia-Pacific region than in other parts of the world. Infectious agents including bacteria, viruses, fungi, and parasites, could lead to ocular inflammation with various chorioretinal and ocular surface manifestations, including optic neuritis, optic disc edema, retinal vasculitis, iritis, chorioretinitis, neuroretinities, conjunctivitis, vitritis, exudative retinal detachment and retinal vascular occlusions (Majumder et al., 2017; Agarwal et al., 2018).

We developed a rat endotoxin-induced uveitis (EIU) model of infectious uveitis, using footpad injection of 1 mg/kg lipopolysaccharide (LPS; Qin et al. 2014) to assess the potential therapeutic effects of GHRHR. At 24-hours after LPS injection, severe hyperemia and edema were observed in the iris. Accumulation of infiltrating Cd43^{+} leucocytes and Cd68^{+} macrophages as well as increases in the pro-inflammatory mediators tumor necrosis factor-alpha (Tnfa), interleukin-6 (Il6) and monocyte chemoattractant protein-1 (Mcp1), were observed in the aqueous humor of EIU rats. Apart from inflammation in the ocular anterior chamber, LPS also induced retinal inflammation with the activation of microglia, astrocytes and Müller glia (Ren et al., 2018). Utilizing our protocols for anti-inflammatory treatments of uveitis, we investigated the treatment mechanisms of GHRH antagonism in the EIU rat model (**Table 2**). Following LPS insult, there was increase in Ghrhr protein expression parallel to the increase in expression levels of pituitary-specific transcription factor-1, *Ghrhr* splice variant 1, *Ghrh* and *Gh* genes. Elevations of Ghrhr and Gh receptor were detected on the epithelia of the iris and ciliary body. Ghrhr was confined to the infiltrating macrophages and leukocytes in the aqueous humor but not the iris stroma. Treatment with GHRH antagonist MIA-602 decreased the LPS-stimulated surges of Gh and Igf1 in the aqueous humor and alleviated intraocular inflammation through reducing the infiltration of macrophages and leukocytes and the production of Tnfa, Il1β and Mcp1 (Qin et al., 2014). In explant cultures of rat ciliary body and iris, LPS caused a substantial increase of Ghrhr expression with elevated expression and secretion of Il1β, Il6, and Nos2 (Ren et al., 2019). In the leukocyte co-culture, expressions of Ghrhr in the iris and ciliary body explant could be further enhanced after LPS treatment. GHRH antagonist MIA-602 suppressed the elevated expression of Il-1β and Il-6. To elucidate the mechanism of GHRH antagonist treatment in the EIU rat model and the iris-ciliary body explant culture, we investigated the inflammatory cascades mediated by GHRHR. We found that NF-κB subunit p65 was phosphorylated in response to LPS stimulation in human ciliary epithelial cells, resulting in transcriptional upregulation of GHRHR (Liang et al., 2020). Using both bioinformatics and experiments, we identified direct interaction between GHRH-R and JAK2 kinase. In the EIU rat model, JAK2 was elevated in the ciliary body and iris, Stat3 was phosphorylated on tyrosine 705 and there were increased levels of pro-inflammatory factors, including Il6, Il17a, Cox2 and Nos2. GHRH antagonist MIA-602 suppressed the phosphorylation of STAT3 and attenuated the expression of pro-inflammatory factors after LPS treatment of human ciliary epithelial cells or iris-ciliary body explant culture. Blocking the GHRHR/JAK2/STAT3 axis with JAK inhibitor ruxolitinib partially alleviated the LPS-induced acute ocular inflammation with reduction of the clinical score, retinal-choroidal thickness, scotopic a- and b-waves and the photopic b-wave in electroretinography (Du et al., 2021), and infiltration of inflammatory cells and proteins to the aqueous humor. Expressions of STAT3 target genes in rat ciliary body and iris and in human ciliary epithelial cells were also repressed (Liang et al., 2020).

These experimental findings indicate that inflammation in the iris and ciliary body involves the activation of GHRH signaling, which affects the recruitment of immune cells and the production of pro-inflammatory cytokines, and thus contributes to EIU pathogenesis. Importantly, GHRH antagonists mediate the GHRHR/JAK2/STAT3 signaling axis and are thus potential therapeutic agents for the treatment of acute ocular inflammation in uveitis. In another reported study in mice treated by LPS, the antagonist MIA-690 and the agonist MR-409 have shown: anti-inflammatory, anti-oxidative and behavioral remedial effects (Recinella et al., 2020). There is therapeutic potential of GHRHR analogs for treating disorders that disrupt the innate immunologic system of the retina, and further investigation is warranted.

Optic nerve injury model

Optic neuropathies can occur in common and serious ocular complications including glaucoma, traumatic injuries, ischemia, and tumors, which are leading causes of visual impairment and irreversible blindness. Different forms of optic neuropathies share a common pathology of degeneration of retinal ganglion cells (RGCs) and subsequent axonal loss. The mammalian retina has low intrinsic regenerative ability. In experimental models, the presence of myelin-associated inhibitors, scar formation at the injury site and lack of trophic support contribute to the failure of regeneration after ON injury (Cen and Ng, 2018). Inflammation contributes to deterioration of ocular function in uveitis, following ON injury. Roles of inflammation are complicated as revealed by animal studies. On one hand, repressing neuroinflammatory events is effective in RGC protection (Yang et al., 2019). However, moderate ocular inflammation induced by lens injury or macrophage generation activated by zymosan could promote neural repair after ON injury (Zhang and Li, 2020).

The ON is a part of the central nervous system formed by projection axons from RGCs (Ribas and Costa, 2017). Unidirectional axonal pathways in the optic nerve are highly susceptible to injury that causes optic neuropathies (Carelli et al., 2017). We have previously used the rat and mouse ON injury models to investigate the mechanisms of neural repair after ON injury, inflammation-promoting RGC survival and axon regeneration. We have shown that CXCL5 (Liu et al., 2021) and SDF-1 (unpublished data) are also involved in inflammation-promoting neural repair after ON injury. Our recent study (Cen et al. 2021) demonstrated that subcutaneous, but not intravitreal, applications of either GHRH agonist MR-409 or antagonist MIA-602 promoted the survival of RGCs after ON injury (**Table 2**) and acute intraocular pressure elevation (unpublished data). Both MR-409 and MIA-602 activated the PI3K/Akt pathway in the retina after ON crush injury, which was related to cyclic adenosine monophosphate (cAMP) elevation and therefore promoted RGC survival pathways (Nakashima et al., 2018). In contrast to our earlier study on human non-pigmented ciliary epithelial cells, in which GHRHR antagonists reduced the LPS-induced acute ocular inflammatory responses via STAT3 pathway (Liang et al., 2020), neither GHRH agonist nor antagonist affect the STAT3 the pathway in RGCs after ON injury (Cen et al., 2021). In mild ocular inflammation, MR-409 enhanced the effects of macrophage activation by zymosan and lens injury while the antagonist MIA-602 reduced the protective effect of zymosan and lens injury on RGCs.

These results together indicated that the GHRHR signaling pathway, in the presence of macrophage infiltration, regulated the ocular inflammatory response for RGC protection after ON injury. One of our previous studies

also demonstrated that antagonist MIA-602 alleviated endotoxin-induced intraocular inflammation by attenuating macrophage and leukocyte infiltration into the anterior chamber and inhibiting inflammatory cytokine secretion (Qin et al., 2014). It would require further investigation to determine how the GHRH agonist and antagonist show different regulatory responses in the presence of infiltrating macrophages, and to determine how these analogs interact with the infiltrating macrophages after ON injury for protection of RGCs. Furthermore, the promotion of RGC survival by the GHRH agonist could also be associated with microglia activation since the agonist MR-409 promoted microglia activation in the retina after ON injury. This is consistent with the capability of activated microglia to protect the brain from traumatic injury and other central nervous system diseases (Liu et al., 2021). Future studies may investigate how microglial activation by GHRH agonists is related to the protection of RGCs.

Potential Clinical Applications

GHRHR is present in many cells and tissues in human. There are potential clinical applications of GHRH analogs (Schally et al., 2019). GHRH agonists and antagonists have shown positive effects for treatment of cancers (Gan et al., 2016; Zarandi et al., 2017), Alzheimer's disease (Jaszberenyi et al., 2012), diabetes (Zhang et al., 2015), dyslipidemia (Romero et al., 2016) and heart diseases (Kanashiro-Takeuchi et al., 2015). Clinical trials have been conducted to enhance immune effects in compromised patients (Khorram et al., 1997), improve mild cognitive impairment (Baker et al., 2012) and decrease serum cholesterol levels in type 2 diabetes (Clemmons et al., 2017). For ocular diseases, there have been investigations in experimental models on anti-inflammatory treatments and neuroprotection of RGCs. GHRH and GHRHR have been identified in different ocular tissues, including corneal epithelium and endothelium, ciliary body, trabecular meshwork, ON, and retina (Dubovy et al., 2017). GHRH antagonists could alleviate ocular inflammation in experimental uveitis (Qin et al., 2014; Ren et al., 2019) and in pterygium (Qin et al., 2018). They should be investigated for treatment of non-infectious ocular inflammation along with glucocorticoids, nonsteroidal anti-inflammatory drugs, immunosuppressors, and biologics. Notably, there is experimental evidence that GHRH agonists and antagonists exerted neuroprotective effects on RGCs. The GHRH agonist MR-409 protected RGCs in early experimental diabetic retinopathy through anti-oxidative and anti-inflammatory activities (Thounaojam et al., 2017). Systematic applications of both GHRH agonist MR-409 and antagonist MIA-602 significantly improved survival of RGCs after ON injury in rats. In mild ocular inflammation conditions, agonist MR-409 further enhanced the promoting effects of macrophage activation, while antagonist MIA-602 suppressed the protective effect of inflammation on RGCs (Cen et al., 2021). We have obtained similar results on maintaining RGC survival in our acute high intraocular pressure model (unpublished data). Accordingly, MIA-602 may exert protective effects in serious inflammation that is detrimental to retinal neurons. We conclude that presence of the GHRH/GH axis is important for the maintenance of RGC survival. GHRH analogs not only could treat ocular inflammatory diseases, but could also treat inflammation adequately for supporting RGC survival.

Conclusions and Perspectives

GHRHR-GHRH signaling plays a role in ocular inflammatory diseases and warrant further experimental and clinical studies for retinal neuroprotection. According to current and previous findings, besides applications for treatments of cancers, diabetes, Alzheimer's disease and heart diseases, GHRHR analogs could also modulate inflammation progression in ocular complications of uveitis and optic nerve injuries. Reported experimental evidence as summarized in this review provides the proof of concept for the use of GHRHR agonists and antagonists for clinical therapy of uveitis and ON injury. Further studies are required to evaluate the effects of GHRHR agonists and antagonists in other experimental ocular inflammation models and to delineate specific signaling pathways that are involved in the regulation of inflammation and supporting retinal neuronal survival. The progress in understanding of GHRH agonists and antagonists improves prospects of clinical applications in the treatment of ocular inflammation and related complications.

Acknowledgements: *The authors are grateful to Dr. Larry Baum for proofreading of the manuscript.*
Author contributions: *Conception and design: CPP; literature search: LPC, TKN, WKC and CPP; manuscript writing: LPC, TKN, WKC and CPP. All authors approved the final manuscript.*
Conflicts of interest: *The authors declare no conflicts of interest.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Open peer reviewer: *Nathan K Evanson, Cincinati Children's Hospital Medical Center, USA.*
Additional file: *Open peer review report 1.*

References

Agarwal A, Aggarwal K, Gupta V (2019) Infectious uveitis: an Asian perspective. Eye (Lond) 33:50-65.
Baker LD, Barsness SM, Borson S, Merriam GR, Friedman SD, Craft S, Vitiello MV (2012) Effects of growth hormone-releasing hormone on cognitive function in adults with mild cognitive impairment and healthy older adults: results of a controlled trial. Arch Neurol 69:1420-1429.
Barabutis N, Schally AV (2010) Growth hormone-releasing hormone Extrapituitary effects in physiology and pathology. Cell Cycle 9:4110-4116.
Biscaldi I, Fiori A, Moretta A, Valtorta A (1990) Efficacia del GH-releasing hormone e del GH-biosintetico in un gruppo di bambini con deficit di ormone della crescita. Medicina (Firenze) 10:145-146.
Bloch B, Brazeau P, Bloom F, Ling N (1983) Topographical study of the neurons containing hpGRF immunoreactivity in monkey hypothalamus. Neurosci Lett 37:23-28.
Cai R, Schally AV, Cui T, Szalontay L, Halmos G, Sha W, Kovacs M, Jaszberenyi M, He J, Rick FG, Popovics P, Kanashiro-Takeuchi R, Hare JM, Block NL, Zarandi M (2014) Synthesis of new potent agonistic analogs of growth hormone-releasing hormone (GHRH) and evaluation of their endocrine and cardiac activities. Peptides 52:104-112.
Carelli V, La Morgia C, Ross-Cisneros FN, Sadun AA (2017) Optic neuropathies: the tip of the neurodegeneration iceberg. Hum Mol Genet 26:R139-R150.
Cen LP, Liu YF, Ng TK, Luo JM, van Rooijen N, Zhang M, Pang CP, Cui Q (2018) Casein kinase-II inhibition promotes retinal ganglion cell survival and axonal regeneration. Exp Eye Res 177:153-159.
Cen LP, Ng TK (2018) Stem cell therapy for retinal ganglion cell degeneration. Neural Regen Res 13:1352-1353.
Cen LP, Ng TK, Liang JJ, Xu C, Zhuang X, Liu YF, Chen SL, Xu Y, Yang Q, Yuan XL, Qin YJ, Chan SO, Chen H, Zhang M, Schally AV, Pang CP (2021) Agonist of growth hormone-releasing hormone enhances retinal ganglion cell protection induced by macrophages after optic nerve injury. Proc Natl Acad Sci U S A 118:e1920834118.
Chu WK, Law KS, Chan SO, Yam JC, Chen LJ, Zhang H, Cheung HS, Block NL, Schally AV, Pang CP (2016) Antagonists of growth hormone-releasing hormone receptor induce apoptosis specifically in retinoblastoma cells. Proc Natl Acad Sci U S A 113:14396-14401.
Clemmons DR, Miller S, Mamputu JC (2017) Safety and metabolic effects of tesamorelin, a growth hormone-releasing factor analogue, in patients with type 2 diabetes: A randomized, placebo-controlled trial. PLoS One 12:e0179538.
Cohen E, Belkacem S, Fedala S, Collot N, Khallouf E, Dastot F, Polak M, Duquesnoy P, Brioude F, Rose S, Viot G, Soleyan A, Carel JC, Sobrier ML, Chanson P, Gatelais F, Heinrichs C, Kaffel N, Coutant R, Savaş Erdeve Ş, et al. (2019) Contribution of functionally assessed GHRHR mutations to idiopathic isolated growth hormone deficiency in patients without GH1 mutations. Hum Mutat 40:2033-2043.
Coy DH, Hocart SJ, Murphy WA (1991) Human growth hormone-releasing hormone analogues with much improved in vitro growth hormone-releasing potencies in rat pituitary cells. Eur J Pharmacol 204:179-185.
DiToro D, Harbour SN, Bando JK, Benavides G, Witte S, Laufer VA, Moseley C, Singer JR, Frey B, Turner H, Bruning J, Darley-Usmar V, Gao M, Conover C, Hatton RD, Frank S, Colonna M, Weaver CT (2020) Insulin-like growth factors are key regulators of T helper 17 regulatory T cell balance in autoimmunity. Immunity 52:650-667.
Du L, Yip YWY, Ng HK, Ho BM, He JN, Chan SO, Pang CP, Chu WK (2021) Ruxolitinib alleviates uveitis caused by salmonella typhimurium endotoxin. Microorganisms 9:1481.
Dubovy S, Echegaray JJ, Fernandez M, Block NL, Unoki N, Perez R, Vidaurre I, Lee R, Mehrdad N, Schally AV (2017) Expression of hypothalamic neurohormones and their receptors in the human eye. Oncotarget 8:66796-66814.
Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjöstedt E, Lundberg E, Szigyarto CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, et al. (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 13:397-406.
Felix AM, Heimer EP, Wang CT, Lambros TJ, Fournier A, Mowles TF, Maines S, Campbell RM, Wegrzynski BB, Toome V, Fry D, Madison VS (1988) Synthesis, biological activity and conformational analysis of cyclic GRF analogs. Int J Pept Protein Res 32:441-454.
Florea V, Majid SS, Kanashiro-Takeuchi RM, Cai RZ, Block NL, Schally AV, Hare JM, Rodrigues CO (2014) Agonists of growth hormone-releasing hormone stimulate self-renewal of cardiac stem cells and promote their survival. Proc Natl Acad Sci U S A 111:17260-17265.
Friedman SD, Baker LD, Borson S, Jensen JE, Barsness SM, Craft S, Merriam GR, Otto RK, Novotny EJ, Vitiello MV (2013) Growth hormone-releasing hormone effects on brain γ-aminobutyric acid levels in mild cognitive impairment and healthy aging. JAMA Neurol 70:883-890.
Gan J, Ke X, Jiang J, Dong H, Yao Z, Lin Y, Lin W, Wu X, Yan S, Zhuang Y, Chu WK, Cai R, Zhang X, Cheung HS, Block NL, Pang CP, Schally AV, Zhang H (2016) Growth hormone-releasing hormone receptor antagonists inhibit human gastric cancer through downregulation of PAK1-STAT3/NF-kappaB signaling. Proc Natl Acad Sci U S A 113:14745-14750.
Gaudreau P, Boulanger L, Abribat T (1992) Affinity of human growth hormone-releasing factor (1-29)NH2 analogues for GRF binding sites in rat adenopituitary. J Med Chem 35:1864-1869.
Gesmundo I, Miragoli M, Carullo P, Trovato L, Larcher V, Di Pasquale E, Brancaccio M, Mazzola M, Villanova T, Sorge M, Taliano M, Gallo MP, Alloatti G, Penna C, Hare JM, Ghigo E, Schally AV, Condorelli G, Granata R (2017) Growth hormone-releasing hormone attenuates cardiac hypertrophy and improves heart function in pressure overload-induced heart failure. Proc Natl Acad Sci U S A 114:12033-12038.
Hsu YR, Huang JC, Tao Y, Kaburaki T, Lee CS, Lin TC, Hsu CC, Chiou SH, Hwang DK (2019) Noninfectious uveitis in the Asia-Pacific region. Eye (Lond) 33:66-77.
Icyuz M, Fitch M, Zhang F, Challa A, Sun LY (2020) Physiological and metabolic features of mice with CRISPR/Cas9-mediated loss-of-function in growth hormone-releasing hormone. Aging (Albany NY) 12:9761-9780.
Izdebski J, Pinski J, Horvath JE, Halmos G, Groot K, Schally AV (1995) Synthesis and biological evaluation of superactive agonists of growth hormone-releasing hormone. Proc Natl Acad Sci U S A 92:4872-4876.
Jaszberenyi M, Rick FG, Szalontay L, Block NL, Zarandi M, Cai RZ, Schally AV (2012) Beneficial effects of novel antagonists of GHRH in different models of Alzheimer's disease. Aging (Albany NY) 4:755-767.

Kale S, Gada JV, Jadhav S, Lila AR, Sarathi V, Budyal S, Patt H, Goroshi MR, Thadani PM, Arya S, Kamble AA, Patil VA, Acharya S, Sankhe S, Shivane V, Raghavan V, Bandgar TR, Shah NS (2020) Genetic spectrum and predictors of mutations in four known genes in Asian Indian patients with growth hormone deficiency and orthotopic posterior pituitary: an emphasis on regional genetic diversity. Pituitary 23:701-715.

Kanashiro-Takeuchi RM, Takeuchi LM, Rick FG, Dulce R, Treuer AV, Florea V, Rodrigues CO, Paulino EC, Hatzistergos KE, Selem SM, Gonzalez DR, Block NL, Schally AV, Hare JM (2012) Activation of growth hormone releasing hormone (GHRH) receptor stimulates cardiac reverse remodeling after myocardial infarction (MI). Proc Natl Acad Sci U S A 109:559-563.

Kanashiro-Takeuchi RM, Szalontay L, Schally AV, Takeuchi LM, Popovics P, Jaszberenyi M, Vidaurre I, Zarandi M, Cai RZ, Block NL, Hare JM, Rick FG (2015) New therapeutic approach to heart failure due to myocardial infarction based on targeting growth hormone-releasing hormone receptor. Oncotarget 6:9728-9739.

Khorram O, Yeung M, Vu L, Yen SS (1997) Effects of [norleucine27]growth hormone-releasing hormone (GHRH) (1-29)-NH2 administration on the immune system of aging men and women. J Clin Endocrinol Metab 82:3590-3596.

Kiaris H, Chatzistamou I, Papavassiliou AG, Schally AV (2011) Growth hormone-releasing hormone: not only a neurohormone. Trends Endocrinol Metab 22:311-317.

Klukovits A, Schally AV, Szalontay L, Vidaurre I, Papadia A, Zarandi M, Varga JL, Block NL, Halmos G (2012) Novel antagonists of growth hormone-releasing hormone inhibit growth and vascularization of human experimental ovarian cancers. Cancer 118:670-680.

Kopelman PG, Noonan K, Goulton R, Forrest AJ (1985) Impaired growth hormone response to growth hormone releasing factor and insulin-hypoglycaemia in obesity. Clin Endocrinol (Oxf) 23:87-94.

Kovacs M, Gulyas J, Bajusz S, Schally AV (1988) An evaluation of intravenous, subcutaneous, and in vitro activity of new agmatine analogs of growth hormone-releasing hormone hGH-RH (1-29)NH2. Life Sci 42:27-35.

Leone S, Recinella L, Chiavaroli A, Ferrante C, Orlando G, Vacca M, Salvatori R, Brunetti L (2018) Behavioural phenotyping, learning and memory in young and aged growth hormone-releasing hormone-knockout mice. Endocr Connect 7:924-931.

Li J, Yip YWY, Ren J, Hui WK, He JN, Yu QX, Chu KO, Ng TK, Chan SO, Pang CP, Chu WK (2019) Green tea catechins alleviate autoimmune symptoms and visual impairment in a murine model for human chronic intraocular inflammation by inhibiting Th17-associated pro-inflammatory gene expression. Sci Rep 9:2301.

Liang WC, Ren JL, Yu QX, Li J, Ng TK, Chu WK, Qin YJ, Chu KO, Schally AV, Pang CP, Chan SO (2020) Signaling mechanisms of growth hormone-releasing hormone receptor in LPS-induced acute ocular inflammation. Proc Natl Acad Sci U S A 117:6067-6074.

Liao F, Zhang TJ, Mahan TE, Jiang H, Holtzman DM (2015) Effects of growth hormone-releasing hormone on sleep and brain interstitial fluid amyloid-β in an APP transgenic mouse model. Brain Behav Immun 47:163-171.

Liu YF, Liang JJ, Ng TK, Hu Z, Xu C, Chen S, Chen SL, Xu Y, Zhuang X, Huang S, Zhang M, Pang CP, Cen LP (2021) CXCL5/CXCR2 modulates inflammation-mediated neural repair after optic nerve injury. Exp Neurol 341:113711.

Majumder PD, Ghosh A, Biswas J (2017) Infectious uveitis: An enigma. Middle East Afr J Ophthalmol 24:2-10.

Mayo KE (1992) Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. Mol Endocrinol 6:1734-1744.

Mullis PE (2011) Genetics of GHRH, GHRH-receptor, GH and GH-receptor: its impact on pharmacogenetics. Best Pract Res Clin Endocrinol Metab 25:25-41.

Murialdo G, Zerbi F, Filippi U, Tosca P, Fonzi S, Di Paolo E, Costelli P, Porro S, Polleri A, Savoldi F (1990-1991) Cholinergic modulation of growth hormone-releasing hormone effects on growth hormone secretion in dementia. Neuropsychobiology 24:129-134.

Nakashima KI, Iwao K, Inoue T, Haga A, Tsutsumi T, Mochita MI, Fujimoto T, Tanihara H (2018) Stimulation of the adenosine A3 receptor, not the A1 or A2 receptors, promote neurite outgrowth of retinal ganglion cells. Exp Eye Res 170:160-168.

Nemeroff CB, Krishnan KR, Belkin BM, Ritchie JC, Clark C, Vale WW, Rivier JE, Thorner MO (1989) Growth hormone response to growth hormone releasing factor in Alzheimer's disease. Neuroendocrinology 50:663-666.

Netchine I, Talon P, Dastot F, Vitaux F, Goossens M, Amselem S (1998) Extensive phenotypic analysis of a family with growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. J Clin Endocrinol Metab 83:432-436.

Pan WW, Lin F, Fort PE (2021) The innate immune system in diabetic retinopathy. Prog Retin Eye Res 84:100940.

Pérez Jurado LA, Phillips JA 3rd, Francke U (1994) Exclusion of growth hormone (GH)-releasing hormone gene mutations in familial isolated GH deficiency by linkage and single strand conformation analysis. J Clin Endocrinol Metab 78:622-628.

Qin YJ, Chan SO, Chong KK, Li BF, Ng TK, Yip YW, Chen H, Zhang M, Block NL, Cheung HS, Schally AV, Pang CP (2014) Antagonist of GH-releasing hormone receptors alleviates experimental ocular inflammation. Proc Natl Acad Sci U S A 111:18303-18308.

Qin YJ, Chu WK, Huang L, Ng CHY, Chan TCY, Cao D, Yang C, Zhang L, Huang SP, Li J, Lin HL, Li WQ, Chen L, Schally AV, Chan SO, Zhang HY, Pang CP (2018) Induction of apoptosis in pterygium cells by antagonists of growth hormone-releasing hormone receptors. Invest Ophthalmol Vis Sci 59:5060-5066.

Qin YJ, Chan SO, Lin HL, Zhang YQ, He BT, Zhang L, Yu HH, Chu WK, Pang CP, Zhang HY (2020) Increased expression of growth hormone-releasing hormone in fibrinous inflammation of proliferative diabetic retinopathy. Am J Ophthalmol 215:81-90.

Rathnasamy G, Foulds WS, Ling EA, Kaur C (2017) Glutamate inhibits the pro-survival effects of insulin-like growth factor-1 on retinal ganglion cells in hypoxic neonatal rat retina. Mol Neurobiol 54:3453-3464.

Recinella L, Chiavaroli A, Orlando G, Ferrante C, Marconi GD, Gesmundo I, Granata R, Cai R, Sha W, Schally AV, Brunetti L, Leone S (2020) Antinflammatory, antioxidant, and behavioral effects induced by administration of growth hormone-releasing hormone analogs in mice. Sci Rep 10:732.

Recinella L, Chiavaroli A, Orlando G, Ferrante C, Gesmundo I, Granata R, Cai R, Sha W, Schally AV, Brunetti L, Leone S (2021) Growth hormone-releasing hormone antagonistic analog MIA-690 stimulates food intake in mice. Peptides 142:170582.

Ren JL, Yu QX, Liang WC, Leung PY, Ng TK, Chu WK, Pang CP, Chan SO (2018) Green tea extract attenuates LPS-induced retinal inflammation in rats. Sci Rep 8:429.

Ren JL, Yu QX, Ma D, Liang WC, Leung PY, Ng TK, Chu WK, Schally AV, Pang CP, Chan SO (2019) Growth hormone-releasing hormone receptor mediates cytokine production in ciliary and iris epithelial cells during LPS-induced ocular inflammation. Exp Eye Res 181:277-284.

Ribas VT, Costa MR (2017) Gene manipulation strategies to identify molecular regulators of axon regeneration in the central nervous system. Front Cell Neurosci 11:231.

Robberecht P, Coy DH, Waelbroeck M, Heiman ML, de Neef P, Camus JC, Christophe J (1985) Structural requirements for the activation of rat anterior pituitary adenylate cyclase by growth hormone-releasing factor (GRF): discovery of (N-Ac-Tyr1, D-Arg2)-GRF(1-29)-NH2 as a GRF antagonist on membranes. Endocrinology 117:1759-1764.

Romero MJ, Lucas R, Dou H, Sridhar S, Czikora I, Mosieri EM, Rick FG, Block NL, Sridhar S, Fulton D, Weintraub NL, Bagi Z, Schally AV (2016) Role of growth hormone-releasing hormone in dyslipidemia associated with experimental type 1 diabetes. Proc Natl Acad Sci U S A 113:1895-1900.

Rübsam A, Parikh S, Fort PE (2018) Role of inflammation in diabetic retinopathy. Int J Mol Sci 19:942.

Sato K, Hotta M, Kageyama J, Chiang TC, Hu HY, Dong MH, Ling N (1987) Synthesis and in vitro bioactivity of human growth hormone-releasing factor analogs substituted with a single D-amino acid. Biochem Biophys Res Commun 149:531-537.

Schally AV, Bowers CY (1964) Corticotropin-releasing factor and other hypothalamic peptides. Metabolism 13:1190-1205.

Schally AV, Muller EE, Sawano S (1968) Effect of porcine growth hormone-releasing factor on the release and synthesis of growth hormone in vitro. Endocrinology 82:271-276.

Schally AV, Sawano S, Arimura A, Barrett JF, Wakabayashi I, Bowers CY (1969) Isolation of growth hormone-releasing hormone (GRH) from porcine hypothalami. Endocrinology 84:1493-1506.

Schally AV, Arimura A, Bowers CY, Wakabayashi I, Kastin AJ, Redding TW, Mittler JC, Nair RM, Pizzolato P, Segal AJ (1970) Purification of hypothalamic releasing hormones of human origin. J Clin Endocrinol Metab 31:291-300.

Schally AV, Zhang X, Cai R, Hare JM, Granata R, Bartoli M (2019) Actions and potential therapeutic applications of growth hormone-releasing hormone agonists. Endocrinology 160:1600-1612.

Schwander JC, Hauri C, Zapf J, Froesch ER (1983) Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. Endocrinology 113:297-305.

Shrihari TG (2017) Dual role of inflammatory mediators in cancer. Ecancermedicalscience 11:721.

Shohreh R, Pardo CA, Guaraldi F, Schally AV, Salvatori R (2011) GH, but not GHRH, plays a role in the development of experimental autoimmune encephalomyelitis. Endocrinology 152:3803-3810.

Srivastava CH, Monts BS, Rothrock JK, Peredo MJ, Pescovitz OH (1995) Presence of a spermatogenic-specific promoter in the rat growth hormone-releasing hormone gene. Endocrinology 136:1502-1508.

Sun LY, Spong A, Swindell WR, Fang Y, Hill C, Huber JA, Boehm JD, Westbrook R, Salvatori R, Bartke A (2013) Growth hormone-releasing hormone disruption extends lifespan and regulates response to caloric restriction in mice. Elife 2:e01098.

Tamaki K, Usui-Ouchi A, Murakami A, Ebihara N (2016) Fibrocytes and fibrovascular membrane formation in proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci 57:4999-5005.

Telegdy G, Tanaka M, Schally AV (2011) Effects of the growth hormone-releasing hormone (GH-RH) antagonist on brain functions in mice. Behav Brain Res 224:155-158.

Thorner MO, Reschke J, Chitwood J, Rogol AD, Furlanetto R, Rivier J, Vale W, Blizzard RM (1985) Acceleration of growth in two children treated with human growth hormone-releasing factor. N Engl J Med 312:4-9.

Thounaojam MC, Powell FL, Patel S, Gutsaeva DR, Tawfik A, Smith SB, Nussbaum J, Block NL, Martin PM, Schally AV, Bartoli M (2017) Protective effects of agonists of growth hormone-releasing hormone (GHRH) in early experimental diabetic retinopathy. Proc Natl Acad Sci U S A 114:13248-13253.

Tunyasuvunakool K, Adler J, Wu Z, Green T, Zielinski M, Žídek A, Bridgland A, Cowie A, Meyer C, Laydon A, Velankar S, Kleywegt GJ, Bateman A, Evans R, Pritzel A, Figurnov M, Ronneberger O, Bates R, Kohl SAA, Potapenko A, et al. (2021) Highly accurate protein structure prediction for the human proteome. Nature 596:590-596.

Vitiello MV, Moe KE, Merriam GR, Mazzoni G, Buchner DH, Schwartz RS (2006) Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiol Aging 27:318-323.

Waelbroeck M, Robberecht P, Coy DH, Camus JC, De Neef P, Christophe J (1985) Interaction of growth hormone-releasing factor (GRF) and 14 GRF analogs with vasoactive intestinal peptide (VIP) receptors of rat pancreas. Discovery of (N-Ac-Tyr1,D-Phe2)-GRF(1-29)-NH2 as a VIP antagonist. Endocrinology 116:2643-2649.

Winston CN, Goetzl EJ, Baker LD, Vitiello MV, Rissman RA (2018) Growth hormone-releasing hormone modulation of neuronal exosome biomarkers in mild cognitive impairment. J Alzheimers Dis 66:971-981.

Xiang P, Jing W, Lin Y, Liu Q, Shen J, Hu X, Chen J, Cai R, Hare JM, Zhu W, Schally AV, Yu H (2021) Improvement of cardiac and systemic function in old mice by agonist of growth hormone-releasing hormone. J Cell Physiol doi: 10.1002/jcp.30490.

Xiong X, Ke X, Wang L, Yao Z, Guo Y, Zhang X, Chen Y, Pang CP, Schally AV, Zhang H (2020) Splice variant of growth hormone-releasing hormone receptor drives esophageal squamous cell carcinoma conferring a therapeutic target. Proc Natl Acad Sci U S A 117:6726-6732.

Yang Y, Xu C, Chen Y, Liang JJ, Xu Y, Chen SL, Huang S, Yang Q, Cen LP, Pang CP, Sun XH, Ng TK (2019) Green tea extract ameliorates ischemia-induced retinal ganglion cell degeneration in rats. Oxid Med Cell Longev 2019:8407206.

Zarandi M, Horvath JE, Halmos G, Pinski J, Nagy A, Groot K, Rekasi Z, Schally AV (1994) Synthesis and biological activities of highly potent antagonists of growth hormone-releasing hormone. Proc Natl Acad Sci U S A 91:12298-12302.

Zarandi M, Cai R, Kovacs M, Popovics P, Szalontay L, Cui T, Sha W, Jaszberenyi M, Varga J, Zhang X, Block NL, Rick FG, Halmos G, Schally AV (2017) Synthesis and structure-activity studies on novel analogs of human growth hormone releasing hormone (GHRH) with enhanced inhibitory activities on tumor growth. Peptides 89:60-70.

Zhang L, Li X (2020) Toll-like receptor-9 (TLR-9) deficiency alleviates optic nerve injury (ONI) by inhibiting inflammatory response in vivo and in vitro. Exp Cell Res 396:112159.

P-Reviewer: Evanson NK; C-Editors: Zhao M, Liu WJ; Wang Lu; T-Editor: Jia Y