Supporting Information

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SI Materials and Methods

Preparation of Peptides. The peptide analog GHRH-R antagonist MIA-602 and the GHRH-R agonist MR-409 were prepared in our laboratory (Endocrine, Polypeptide, and Cancer Institute, Miami Veterans Affairs Medical Center) by solid-phase synthesis using Boc-chemistry and were purified by reversed-phase HPLC. The chemical structure of MIA-602 is [PhAc-Ada0, D-Arg^{2}, Fpa5^{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}]hGH-RH(1-29)NH2. The structure of the GHRH-R agonist MR-409 is [*N*-Me-Tyr^{1}, D-Ala^{2}, Orn^{12}, Abu^{15}, Orn^{21}, Nle^{27}, Asp^{28}] hGH-RH(1-29)NH-CH3. Abbreviations for the noncoded amino acids and acyl groups used in the peptides are Abu, α-aminobutyric acid; Ada, 12-aminododecanoic acid; Agm, agmatine; Cpa, para-chloro-phenylalanine; Fpa5, pentafluoro-phenylalanine; Har, homoarginine; Me-Ala, *N*-methyl-alanine; Nle, norleucine; Orn, ornithine; PhAc, phenylacetyl; *N*-Me-Tyr, *N*-methyl-tyrosine.

Immunostaining. For immunostaining, the slides were heated in a pressure cooker (Biocare Medical) to induce epitope retrieval. Primary antibodies used included rabbit polyclonal antibody to GHRH-R (1:80 dilution; ab28692; Abcam Inc.); mouse anti-rat monoclonal antibodies against CD43 (1:80 dilution; AbD Serotec) and CD68 (1:100 dilution; AbD Serotec); goat polyclonal antibodies to GHR and GH (1:20 dilution; sc-10351 and sc-10364, respectively; Santa Cruz Biotechnology, Inc.), and mouse anti-IGF1 (1:50 dilution; clone Sm1.2, 05-172; EMD Millipore). After blocking with 0.1% bovine serum at room temperature for 1 h, primary antibody was applied to the sections and incubated at 4 °C overnight, followed by incubation with secondary antibodies (Alexa Fluor 488 or 594; 1:1,000 dilution; Invitrogen) at room temperature for 1 h before nuclear counterstaining with DAPI (1:2,000 dilution). For double staining of the ocular tissues, GHRH-R–CD43, GHRH-R–CD68, and GHR-IGF1 were added to the slides, followed by Alexa Fluor 488 and Alexa Fluor 594. The sections were mounted using aqueous mounting medium (GBI Labs) and examined under a fluorescence microscope (Diagnostic Instruments). Control sections were processed as above but without primary antibody. The primary antibodies used were rabbit polyclonal antibody to GHRH-R (1:80 dilution; ab28692; Abcam Inc.); mouse anti-rat monoclonal antibodies against CD43 (1:80 dilution; AbD Serotec) and CD68 (1:100 dilution; AbD Serotec); goat polyclonal to GHR and GH (1:20 dilution; sc-10351 and sc-10364, respectively; Santa Cruz Biotechnology), and mouse anti-IGF1 (1:50 dilution; clone Sm1.2, 05-172; EMD Millipore).

Western Blotting Analysis. For Western blotting analysis, proteins in the gel were transferred onto nitrocellulose membrane (Amersham Hybond-ECL; GE Healthcare), blocked with 5% nonfat dried milk in Tris-buffer saline (TBS) for 1 h at room temperature to saturate the excess protein-binding sites, followed by incubation with GHRH-R antibody (1:3,000 dilution; ab28692; Abcam Inc.) with shaking at 4 °C overnight. The membrane was washed three times in TBS-0.05% Tween-20 (TBST) before incubation with HRP-coupled secondary antibodies (1:3,000; Santa Cruz) for 1 h at room temperature, followed by three washes with TBST. The immunoreactive bands were visualized with enhanced chemiluminescence reagents (GE Healthcare) in the Universal Hood II image system (Bio-Rad Laboratories). Band intensities of GHRH-R were normalized to the intensities of housekeeping β-actin using Image J software v. 1.47 (National Institutes of Health). Fold changes in GHRH-R protein expression resulting from LPS insult were obtained for each tissue by comparing these normalized values with the corresponding values in the control group.

RT-PCR. RT-PCR was performed in 25-μL reaction mixtures in a cycling program: an initial step for activation of the Platinum Taq DNA polymerase (Invitrogen) at 95 °C for 2 min followed by 38 cycles of denaturation at 92 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and extension at 72 °C for 5 min. After amplification, the DNA products were electrophoresed in 2.0% agarose gel.

qRT-PCR. qRT-PCR was performed with a SYRB Green PCR mixture containing 10 μL of 2× 480 SYRB Green I Master (Roche), 0.5 μL of 10 nM primer (Invitrogen), 1 μL cDNA, and 8 μL of double-distilled water. The thermal cycle was preincubation 95 °C for 10 min, 40 amplification cycles with denaturation at 95 °C for 15 s, annealing/extension at 60 °C for 1 min. The relative Pit-1, CBP, GHRH-R, SV-1, GHRH, and GH mRNA expression of each sample was calculated as described (1). The threshold cycle value of the target gene ($C_{Ttarget}$) was corrected with that of the internal control gene GAPDH (C_{TGAPDH}). The expression values in each gene [$2^{-(CTtarget - CTGAPDH)}$] were normalized. Fold changes resulting from treatment were obtained by comparing the normalized expression values for each gene in the treatment group and in the LPS group.

1. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 3(6):1101–1108.

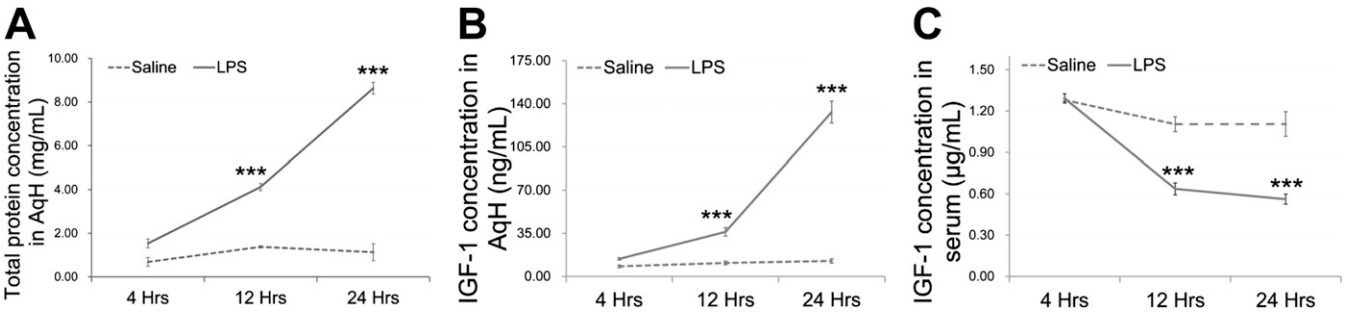

Fig. S1. Total protein and IGF1 concentration in aqueous humor. (*A*) Total protein concentration in aqueous humor (AqH) at 4, 12, and 24 h after LPS injection. (*B*) IGF1 concentration in AqH at 4, 12, and 24 h after LPS injection. (*C*) IGF1 concentration in serum at 4, 12, and 24 h after LPS injection. ***$P < 0.01$, compared with the normal control (saline+saline); Mann–Whitney test; $n = 6$ at each time point.

Table S1. Gene-specific primers for rat Pit-1, CBP, GHRH-R, SV-1, GHRH, GH, and GAPDH

Gene	Identification	Forward, 5′–3′	Reverse, 5′–3′	Product size, bp
Pit-1	NM_013008.3	AGCAGTTTGCCAACGAATTT	CCTCCAGCCACTTGGATAAA	189
CBP	NM_133381.3	GGACTCCCCTACATGAACCA	CGGCTGCTGATCTGTTGTTA	162
GHRH-R	NM_012850.1	CACTGCCCCAGGAACTACAT	AGCCAGCTGAAGTTGGTCAT	188
SV-1	—	TGGGGAGAGGGAAGGAGTTGT	GCGAGAACCAGCCACCAGAA	523
GHRH	NM_031577.1	CCAATTATATGCCCGCAAAC	GCTGAAAGCTTCATCCTTGG	171
GH	NM_001034848.2	CTGGCTGCTGACACCTACAA	GAAGCGAAGCAATTCCATGT	165
GAPDH	NM_017008.4	GTGCCAGCCTCGTCTCATA	GTTGAACTTGCCGTGGGTAG	190

Table S2. Fold change in total protein and IGF1 concentrations caused by LPS insult

Sample	Value at baseline, μg/L	Value at 24 h, μg/L	Fold change
Total protein in aqueous humor	1.06 ± 0.20	8.63 ± 0.52	8
IGF1 in aqueous humor	10.55 ± 1.45	133.23 ± 8.89	13
IGF1 in serum	1.16 ± 0.05	0.56 ± 0.04	−2

Values at baseline and at 24 h are shown as mean ± SEM. Fold changes were obtained by comparing the value at 24 h with the baseline value.

Table S3. Normalized expression value and fold change in gene expression in the iris-CB after treatment with LPS

Group	*Pit-1* (fold change)	*CBP* (fold change)	*GHRH-R* (fold change)	*SV-1* (fold change)	*GHRH* (fold change)	*GH* (fold change)
Saline+DMSO, $n = 6$	$0.82\times10^{-3} \pm 0.08\times10^{-3}$ (1.0)‡	0.18 ± 0.01 (1.0)‡	$0.37\times10^{-3} \pm 0.04\times10^{-3}$ (1.0)‡	$0.17\times10^{-3} \pm 0.03\times10^{-3}$ (1.0)‡	$0.08\times10^{-3} \pm 0.01\times10^{-3}$ (1.0)‡	$4.28\times10^{-3} \pm 2.59\times10^{-3}$ (1.0)‡
LPS+DMSO, $n = 9$	$8.60\times10^{-3} \pm 3.61\times10^{-3}$ (10.5)††,‡ (1.0)§	0.15 ± 0.01 (−1.2)††,‡ (1.0)§	$2.41\times10^{-3} \pm 0.52\times10^{-3}$ (6.5)†††,‡ (1.0)§	$0.54\times10^{-3} \pm 0.10\times10^{-3}$ (3.2) †††,‡ (1.0)§	$0.54\times10^{-3} \pm 0.12\times10^{-3}$ (6.8)†††,‡ (1.0)§	$5.69\times10^{-3} \pm 1.53\times10^{-3}$ (1.3)‡ (1.0)§
LPS+MIA-602, $n = 9$	$0.22\times10^{-4} \pm 0.03\times10^{-4}$ (−395.7)***,§	0.108 ± 0.004 (−1.4)***,§	$0.14\times10^{-3} \pm 0.01\times10^{-3}$ (−17.3)***,§	$0.40\times10^{-4} \pm 0.04\times10^{-4}$ (−13.6)***,§	$0.20\times10^{-4} \pm 0.07\times10^{-4}$ (−26.4)***,§	$0.65\times10^{-3} \pm 0.36\times10^{-3}$ (−8.8)***,§
LPS+R-409, $n = 9$	$7.13\times10^{-3} \pm 1.65\times10^{-3}$ (−1.2)§	0.146 ± 0.009 (−1.0)§	$4.67\times10^{-3} \pm 1.15\times10^{-3}$ (1.9)§	$4.64\times10^{-3} \pm 1.10\times10^{-3}$ (8.6)***,§	$7.04\times10^{-3} \pm 1.85\times10^{-3}$ (13.0)***,§	$4.40\times10^{-3} \pm 0.67\times10^{-3}$ (-1.3)§
LPS+Dxm, $n = 3$	$0.20\times10^{-3} \pm 0.04\times10^{-3}$ (−42.5)**,§	0.16 ± 0.03 (1.1)§	$0.28\times10^{-3} \pm 0.03\times10^{-3}$ (−8.6)**,§	$0.35\times10^{-3} \pm 0.30\times10^{-3}$ (−1.5)§	$0.27\times10^{-4} \pm 0.09\times10^{-4}$ (−20.5)***,§	$1.7\times10^{-8} \pm 1.5\times10^{-8}$ (−343778.0)***,§

Results are based on qRT-PCR. Values are shown as mean ± SEM. *P* values (Mann–Whitney test) are indicated as $^{**}P < 0.05/3$ compared with the LPS control (LPS+DMSO), Bonferroni adjusted; $^{***}P < 0.01$; $^{\dagger\dagger}P < 0.05/3$ compared with the saline control (saline+DMSO), Bonferroni adjusted; $^{\dagger\dagger\dagger}P < 0.01$. Ratio >1, fold = ratio; ratio <1, fold = −1/ratio.

‡Ratio = normalized expression of each gene in treatment group/normalized expression of each gene in saline control group.

§Ratio = normalized expression of each gene in treatment group/normalized expression of each gene in LPS control group.