An antagonist of growth hormone-releasing hormone protects against LPS-induced increase of bronchoalveolar lavage fluid protein concentration

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Abstract
Growth Hormone-Releasing Hormone (GHRH) is a neuropeptide regulating the release of Growth Hormone (GH) from the anterior pituitary gland, and acts as a growth factor in a diverse variety of tissues. GHRH antagonists (GHRH-Ant) have been developed to counteract those events, and the beneficial effects of those peptides toward homeostasis have been associated with anti-inflammatory activities. Our lab is interested in delineating the mechanisms governing endothelial barrier function. Our goal is to establish new grounds on the development of efficient countermeasures against Acute Respiratory Distress Syndrome (ARDS), which has been associated with thousands of deaths worldwide due to COVID-19. Herein we demonstrate in vivo that GHRH-Ant suppresses LPS-induced increase in bronchoalveolar lavage fluid (BALF) protein concentration, thus protecting the lungs against edema and inflammation.

Keywords Inflammation · Lung · Permeability

Introduction
GHRH is consisted of 44 amino acids [1] and exerts extra-hypothalamic effects in peripheral tissues including brain, heart, and retina. Receptors specific for that hormone—pituitary-type GHRH receptor (GHRH-R) and its splice variants have been detected in various tissues including the lungs, supporting the multifaceted role of GHRH in human physiology [2].

GHRH is associated with inflammatory responses. In human ciliary epithelial cells, NF-κB subunit p65 is phosphorylated by lipopolysaccharides (LPS), resulting in GHRH-R upregulation. GHRH triggers IL-6 release from human peripheral blood mononuclear cells, and enhances immune cell function in the elderly. Moreover, cytokine production in ciliary and iris epithelial cells is mediated by GHRH-R during LPS-induced ocular inflammation [3].

The endothelial barrier regulates the exchange of blood fluid, electrolytes and proteins across the vascular wall. This structure is affected by several stimuli, including LPS, growth factors, and SARS-CoV-2 [4]. Those factors affect several intracellular messengers (kinases, phosphatases) to induce cytoskeletal remodeling by modulating junction and adhesion proteins. Those changes affect endothelial barrier function to increase lung permeability across the endothelial and epithelial barriers [4–6].

Vascular endothelial dysfunction is a central event in the pathogenesis of sepsis and ARDS. Lung hyper-permeability allows fluid, proteins, and macromolecules into the alveolar airspace, resulting in increased BALF protein concentration [7, 8]. GHRH-Ant enhance endothelial barrier function in vitro [9, 10], but their effects in an in vivo model of murine lung injury in the context of BALF protein concentration have not been investigated yet.

Materials and methods
Reagents
GHRH-Ant (#4030691) was purchased from Bachem (Torrance, CA) and LPS from Sigma-Aldrich (St Louis, MO).
The scramble peptide was synthesized in LSU AG Center Biotechnology Lab, Baton Rouge, LA (Invoice No.:1075).

Animals

Seven-week-old C57BL/6 (male) mice were purchased from Envigo (Indianapolis, IN) and maintained in 12:12-h light/dark cycle. The temperature (22–24 °C) and humidity (50–60%) were in control, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental procedures

Mice were treated with intratracheal (I.T.) injection of saline or LPS (1.6 mg/kg) and were post-treated (24 h after LPS) with intravenous (I.V.) (tail vein) injection of either vehicle; or scramble peptide (100 µg/kg); or GHRHaNT (100 µg/kg). Protein concentration was measured by the bicinchoninic acid protein assay kit.

Statistics

The data are expressed as mean values ± SEM (standard error of mean). Student’s t test was used to determine statistically significant differences among groups and GraphPad Prism (version 5.01) to analyze the data.

Results and discussion

Figure 1 suggests that LPS increases BALF protein concentration of LPS-treated mice as compared to the control group. GHRHaNT significantly reduces BALF protein concentration in LPS-pretreated mice, while the scramble peptide for GHRHaNT did not exert any significant effects. Hence, we suggest a protective role of GHRHaNT against LPS-induced injury.

GHRH-R regulates key signaling pathways involved in lung growth and inflammation. GHRHaNT inhibit lung inflammation and fibrosis in bleomycin-induced fibrosis model in vivo and suppress several inflammatory pathways (i.e., ERK, JAK2/STAT3, NF-κB) [9, 11]. They also suppress cofilin and myosin light chain 2 (MLC2) activation to regulate cytoskeletal remodeling in LPS-induced inflammation [12]. Those events increase the transendothelial resistance of endothelial monolayers to enhance barrier integrity [9].

Inflammatory conditions are characterized by excessive generation of reactive oxygen species (ROS) which cause disruption of inter-endothelial junctions, endothelial hyperpermeability and abnormal extravasation of proteinous fluids and macromolecules to the lungs [13]. Those events cause edema formation and infiltration of inflammatory cells. GHRHaNT exert anti-oxidative effects in lung and brain endothelial cells and inhibit lipid and protein oxidation [2]. In contrast, GHRH increases the expression of cyclooxygenase-2, cytochrome c oxidase-IV and inducible nitric oxide synthase, which are involved in ROS generation [2].

Fig. 1 GHRHaNT reduces BALF protein concentration of mice exposed to LPS. Male C57BL/6 mice were treated with an intratracheal (I.T.) injection of saline or LPS (1.6 mg/kg, dissolved in saline), and were post-treated (24 h after LPS) with an intravenous (I.V.) (tail vein) injection of either vehicle (0.1% DMSO in 10% 1,2-propanediol); or scramble peptide (100 µg/kg, dissolved in 0.1% DMSO and 10% 1,2-propanediol); or GHRHaNT (100 µg/kg, dissolved in 0.1% DMSO and 10% 1,2-propanediol). 48 h after LPS, BALF from the lungs was obtained by instilling and withdrawing 1 ml of PBS using tracheal cannula. *P < 0.05 vs LPS, n = 3 animals per group.

Means ± SEM
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Data availability statement The datasets are available from the corresponding author on reasonable request.

Declarations

Conflict of interest None.

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