Available online at www.sciencedirect.com
ScienceDirect
Regulatory Peptides 138 (2007) 113–117
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Effect of ghrelin administration on phagocytic activity in acute cold-restraint stress exposed rats

Cemil Tümer [a,*], Hakkı Murat Bilgin [a], Basra Deniz Obay [a], Hüda Diken [a], Ezel Taşdemir [b], Abdurrahman Şermet [a]

[a] Dicle University Faculty of Medicine, Department of Physiology, 21280 Diyarbakır, Turkey
[b] Developmental Medical Center Diyarbakır, Turkey

Received 21 July 2005; received in revised form 28 August 2006; accepted 31 August 2006
Available online 16 November 2006

Abstract

Ghrelin, an endogenous ligand for growth hormone secretagogue receptor, was identified in the rat stomach. This peptide acts through nitric oxide (NO) by expressing endothelial nitric oxide synthase (eNOS) and down regulating inducible nitric oxide synthase (iNOS) at its gastroprotective effect against restraint stress induced damage. Recently the ghrelin receptor has also been detected in peripheral systems including immune tissue. We have investigated the possible effect of ghrelin on phagocytic activity of peritoneal macrophages in acute cold-restraint stress (ACRS) exposed rats.

The rats were divided into control, stress and ghrelin groups. In ghrelin groups, single dose and three days consecutive dose of ghrelin (20 μg/kg. i.p.) were applied to rats that were exposed to ACRS for 4 h. 1 ml of saline was injected i.p. after ACRS for 3 consecutive days to the rats of the stress groups. Ghrelin administration reduced the increased phagocytic activity induced by ACRS.

We conclude that ghrelin exerts an important role at macrophage phagocytic activity in ACRS exposed rats.

Keywords: Ghrelin; Inducible nitric oxide synthase; Acute cold-restraint stress; Phagocytic activity

1. Introduction

Ghrelin a novel growth hormone releasing peptide, isolated from the x/A like cells of oxyntic mucosa of stomach, has been identified as an endogenous ligand for growth-hormone secretagogue receptor [1–5]. However, ghrelin expression is not restricted to the gastrointestinal tract. Ghrelin is synthesized in the pancreas, kidney, liver, hypothalamus, pituitary and immune cells [6–8]. Like ghrelin, the ghrelin receptor, a G-protein-coupled receptor, has a widespread distribution. Ghrelin receptor is found in the pituitary, hypothalamus, stomach, heart, blood vessels, lung, pancreas, intestine, kidney, adipose tissue, and immune system (B and T cells, neutrophiles) [8–11] but the expression of these receptors at macrophages has not been studied before. The presence of ghrelin and its receptor in a variety of other tissues suggests the paracrine, autocrine and endocrine role of ghrelin. The gastroprotective effect of ghrelin was demonstrated by a lot of investigators. The gastroprotective effect of centrally administered ghrelin appears to be mediated by nitric oxide (NO) pathway [12,13]. NO is the product of conversion of L-arginine to L-citrulline, which is catalyzed by the enzyme NO synthase (NOS) [14,15]. Three isoforms of NOS have been cloned and characterized: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) [16]. NO produced in low levels by the endothelial and neuronal NOS isoforms, functions as a signaling molecule in several biological processes including the regulation of vascular tone and neuronal signaling [17–19]. iNOS is expressed in various cell types, which include vascular smooth muscle cells, hepatocytes, astrocytes, and macrophages and is induced in response to proinflammatory cytokines or bacterial LPS [20–22]. Ghrelin acts through NO by expressing eNOS and down

* Corresponding author. Tel.: +90 412 2488001.
E-mail address: ctumer@dicle.edu.tr (C. Tümer).

0167-0115/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2006.08.010

regulating iNOS at its gastroproprotective effect against restraint stress induced damage [13]. Ghrelin's relation with nNOS at the gastric region in stress conditions could not be shown. Our previous study indicated that increased macrophage phagocytic activity at acute cold-restraint stress (ACRS) exposed rats was induced with iNOS that originated from NO [23].

In the light of the knowledge of ghrelin's down regulating effect on iNOS at gastric smooth muscle, we aimed to investigate if ghrelin has an effect on the phagocytic activity of peritoneal macrophages in ACRS exposed rats.

2. Methods

2.1. Drugs and chemicals

Rat ghrelin and ghrelin EIA kit was purchased from Phoenix Pharmaceuticals, Inc (Phoenix, TX). The chemicals that were used at the measurement of the phagocytic activity and plasma total nitrite levels were purchased from Sigma Chemical Co. (St. Louis, MO, USA).The ghrelin was dissolved in 1 ml of saline and then injected intraperitoneally.

2.2. Animals

Male Wistar rats weighing 200–250 g were divided into 5 groups containing 7 animals each. All the rats housed at under a 12-h dark/light cycle and allowed food and water available ad libitum. The committee for animal experiments of the Dicle University Medical Research Center (Diyarbakýr, Turkey) gave its approval for the project.

All experiments were performed in animals deprived of food for 24 h but allowed free access to tap water 1 h before the experiments. The groups were as follows:

Group 1. Control.
Group 2. (Stress 1). Saline i.p. 30 min before ACRS.
Group 3. (Ghrelin 1). Ghrelin 20 μg/kg i.p. 30 min before ACRS.
Group 4. (Stress 2). Just before administration, 1 ml of saline was injected i.p. after ACRS for 3 consecutive days.
Group 5. (Ghrelin 2). Just before administration, ghrelin 20 μg/kg was injected i.p. after ACRS for 3 consecutive days.

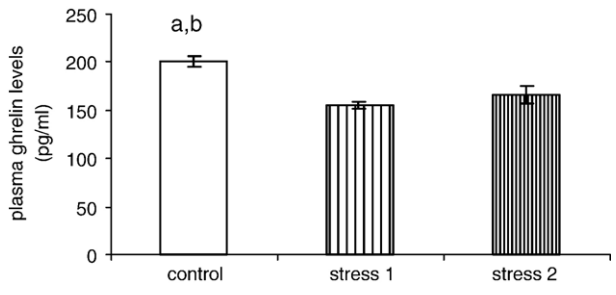

Fig. 1. Effect of ACRS on plasma ghrelin levels. Data represent mean±SE, [a]$p<0.05$ vs. stress 2 and [b]$p<0.01$ vs. stress 1 group.

Table 1
Effects of single dose and three consecutive day/dose of ghrelin (20 μg/kg) administration on phagocytosis number (Pn), phagocytic index (Pi) and total nitrite levels in ACRS exposed rats

	Pn	Pi	Total nitrite
Control	65.0±1.6	1.75±0.10	20.4±0.86
Stress 1	79.8±1.8	2.67±0.11	25.1±1.50
Ghrelin 1	63.4±2.5	1.98±0.11	29.7±1.20
Stress 2	87.4±1.1	2.71±0.10	28.8±0.76
Ghrelin 2	61.7±2.4	2.04±0.14	37.1±2.10

Data are expressed means±SE.

2.3. ACRS protocol

For cold-restraint stress, the rats were restrained in individual, close-fitting tubular wire-mesh cages at 4 °C [24] and the nonstress control group was kept in starvation cages at 22 °C. At the end of 4 h, all the rats were sacrificed.

2.4. Phagocytic activity assays

Peritoneal macrophages were obtained by lavage with a phosphate-buffered saline solution (PBS) (pH=7.2–7.4) were suspended in cold (4 °C) Hanks' Balanced Salt Solution (HBSS) with 5% fetal calf serum (FCS) at an approximate concentration of 2×10^6 cells/ml. The viability of the macrophage was always >95% as determined by trypan blue exclusion. The suspension of macrophages in HBSS was incubated at 37 °C for 30 min in a water bath, with stirring. A suspension of heat killed *Saccharomyces cerevisiae* yeast cells (40×10^6 cells/ml) in HBSS added at the same volume with the macrophage solution was incubated in a separate tube for 60 min. After the incubation, phagocytic activity was evaluated in terms of phagocytosis number (% phagocytic cell in population, macrophages that ingested at least 1 yeast particle) and phagocytosis index (mean number of yeast particles absorbed by one cell) [25] was microscopically enumerated by counting 200 cells (the nonphagocytosing cells too).

2.5. Measurement of plasma levels of total nitrite

Since NO is a very labile molecule, its direct measurement in the biological samples is very difficult [19]. In an aqueous solution, NO reacts with molecular oxygen and accumulates in the plasma as nitrite and nitrate ions. Therefore, the stable oxidation and products of NO, nitrite (NO_2^-) and nitrate (NO_3^-), can be readily measured in biological fluids and have been used in vitro and in vivo as indicators of NO production [26]. Plasma nitrite levels were measured with the Griess reaction [27]. Briefly, samples were initially deproteinized with Somogyi reagent [28]. Total nitrite (nitrite+nitrate) was measured after the reduction of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10^{-8}–10^{-3} mol/l) of sodium nitrite. Linear regression was made by using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as