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ScienceDirect
Life Sciences 80 (2007) 867–872
Life Sciences
www.elsevier.com/locate/lifescie

The effect of centrally administered ghrelin on pituitary ACTH cells and circulating ACTH and corticosterone in rats

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Received 31 July 2006; accepted 4 November 2006

Abstract

Ghrelin is a brain-gut peptide known for its growth hormone (GH)-releasing and appetite-inducing activities. This natural GH secretagogue (GHS) was originally purified from rat stomach, but it is expressed widely in different tissues where it may have endocrine and paracrine effects. The central effects of ghrelin on adrenocorticotropic hormone (ACTH) cells, ACTH release and subsequent corticosterone release from adrenal glands remains to be clarified. The aim of this study was to specifically determine the morphological features of ACTH-producing pituicytes and blood concentration of ACTH and corticosterone after central administration of ghrelin. Five doses of rat ghrelin or PBS ($n=10$ per group) were injected every 24 h (1 μg of ghrelin in 5 μL PBS), into the lateral cerebral ventricle of male rats. Results showed that ghrelin increased ($p<0.05$) absolute and relative pituitary weights compared to controls (58% and 41% respectively). Morphometric parameters, i.e. the volume of the ACTH cells, nuclear volume, and volume density were all increased ($p<0.05$), by 17%, 6% and 13%, respectively, 2 h after the last ghrelin treatment. Ghrelin increased circulating concentrations of ACTH and corticosterone ($p<0.05$) by 62% and 66%, respectively. The data provide clear documentation that intracerebroventricular ghrelin stimulates ACTH cell hypertrophy and proliferation, and promotes ACTH and corticosterone release. Determining the role of ghrelin in physiological stress responses and whether control of the peptide's activity would be useful for prevention and/or treatment of stress-induced diseases remain important research goals.

Keywords: Ghrelin; Intracerebroventricular injection; Pituitary; ACTH cells; Rats; Morphology

Introduction

Ghrelin is a 28-amino acid peptide with an *n*-octanoyl modification on Ser[3], that was originally isolated from rat stomach as a natural ligand of the orphan GH secretagogue receptor type 1a (GHS-R1a) (Kojima et al., 1999). Together with growth hormone-releasing hormone (GHRH) and somatostatin, ghrelin plays a major role in the control of somatotroph function, and it may have an important role in other neuroendocrine functions such as stimulation of lactotroph and corticotroph secretion (Van der Lely et al., 2004). GHS-Rs are expressed in the hypothalamus–pituitary axis as well as in other areas of the central nervous system and in peripheral tissues (Gnanapavan et al., 2002; Volante et al., 2002; Muccioli et al., 2001). It is recognized that ghrelin is widely expressed in different rat and human tissues including pituitary, hypothalamus, bowel, kidney, heart, pancreas, testis, and adrenal cortex (Korbonits and Grossman, 2004), and this wide distribution explains the pleiotropic effects of ghrelin. More specifically, ghrelin exists in rat hypothalamus in two molecular forms: *n*-octanoyl-modified and des-acyl ghrelin (Sato et al., 2005). Together with leptin, ghrelin was mainly considered to be a new major orexigenic factor involved in energy balance and regulation of the neuroendocrine response to fasting and starvation-induced stress (Horvath et al., 2001). One of the central actions of ghrelin was an anxiogenic effect that could be annulled by pretreatment with

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0024-3205/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2006.11.018

Table 1
The effects of intracerebroventricularly applied ghrelin on body weight, absolute and relative pituitary weight in adult male rats

Experimental group	Body weight (g)	Absolute pituitary weight (mg)	Relative pituitary weight (mg/%)
Control	196.6±21.4	5.8±1.5	2.7±0.9
Ghrelin	222. 0±27.3 (+13%)	9.2±1.2 * (+58%)	3.8±0.3 * (+41%)

The values are the means±the standard deviation; $n=10$/group; *$p<0.05$ vs. control.

antagonists of corticotrophin-releasing hormone (CRH) in rodents (Carlini et al., 2004). These results led to investigations of the role of ghrelin in the control of hypothalamo–pituitary–adrenal (HPA) axis in physiological and various pathophysiological conditions.

Before the discovery of ghrelin, moderate but consistent elevation of plasma adrenocorticotropin (ACTH) and serum glucocorticoid levels were reported for a variety of GHSs in different species (Casanueva and Dieguez, 1999; Korbonits and Grossman, 1995; Ghigo et al., 1997; Clark et al., 1997; Thomas et al., 1997; Hickey et al., 1994). In good agreement, it has been recently shown that ghrelin increases ACTH and cortisol release in humans as well as ACTH and corticosterone secretion in rats and mice when administered in what were probably pharmacological doses (Takaya et al., 2000; Wren et al., 2000; Arvat et al., 2001; Asakawa et al., 2001). However, ghrelin administration did not affect ACTH secretion from pituitaries in vitro or in anesthetized animals.

Because the above responses occurred after high doses of either GHS or ghrelin, it was necessary to assess the efficacy after a more "physiological" dosing regimen. This study was specifically designed to evaluate the plasma ACTH and serum corticosterone levels after repetitive central administration of nanomolar doses of ghrelin by the intracerebroventricular (ICV) route in freely moving male rats and correlate these blood concentrations with changes in the immunocytochemical appearance and quantitative morphology of pituitary corticotropes.

Materials and methods

The study was performed on adult male Wistar rats (200±20 g), bred at the Institute of Biomedical Research "Galenika" in Belgrade. They were kept in individual metabolic cages under a 12:12 h light–dark cycle, at 22±2 °C, and were accustomed to daily handling. They were fed a balanced diet for laboratory rats (prepared by "D. D. Veterinarski zavod Subotica", Subotica, Serbia). Food and water were available to rats ad libitum.

Animal preparation

Surgical procedures were performed under ether anesthesia (aether ad narcosis Ph. Iug. III. produced by "Lek", Ljubljana, Slovenia). The rats were implanted with a headset later used for ICV injections. A minimum recovery time of 5 days was permitted before the onset of experiments. The headset consisted of a silastic-sealed 20-gauge cannula (Starčević et al., 1988), implanted into a lateral cerebral ventricle, 1 mm posterior and 1.5 mm lateral to the bregma, and 3 mm below the cortical surface. A small stainless steel anchor screw was placed at a remote site on the skull. The cannula and screw were cemented to the skull with dental acrylic (Simgal; "Galenika", Belgrade, Serbia).

Animal treatment

After recovery, rats were divided into two groups, each containing ten animals. The first group consisted of rats that were treated ICV with 1 μg of rat ghrelin (Global Peptide, lot no. C-et-004) dissolved in 5 μL PBS (phosphate buffered saline) every 24 h for 5 consecutive days. The second group was a control group, comprised of rats treated in the same manner, except that they received only 5 μL of PBS. This dosing regimen was determined from preliminary experiments that showed consistent changes in ACTH cell morphology by day 5. The dose of ICV ghrelin (1 μg) was less than comparable studies using 6.6 μg doses (Wren et al., 2000, 2002). All animals were killed by decapitation under anesthesia 2 h after the last injection. Experimental protocols were approved by the Local Animal Care Committee and conformed to the recommendations given in "Guide for the Care and Use of Laboratory Animals" (1996 National Academy Press, Washington DC).

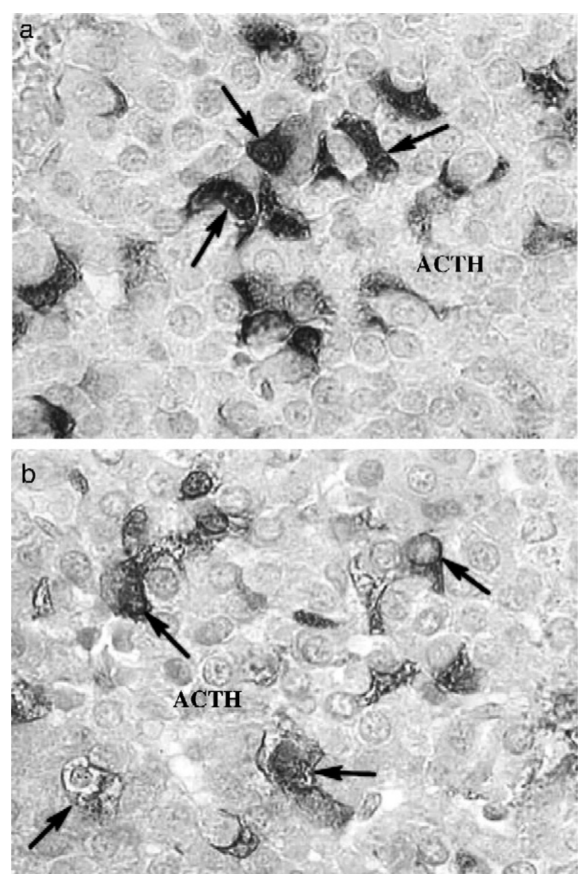

Fig. 1. Typical appearance of immunohistochemically labeled ACTH cells (arrows) in: 1a. control rats, 1b. ghrelin-treated rats (PAP, bar 10 μm).