Available online at www.sciencedirect.com

SCIENCE DIRECT

Regulatory Peptides 125 (2005) 145–149

REGULATORY PEPTIDES

www.elsevier.com/locate/regpep

The effect of agouti-related protein on growth hormone secretion in adult male rats

Hideki Tamura, Jun Kamegai*, Takako Shimizu, Shinya Ishii, Hitoshi Sugihara, Shinichi Oikawa

Department of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-Ku, Tokyo 113-8603, Japan

Received 13 May 2004; accepted 19 August 2004
Available online 15 September 2004

Abstract

Agouti-related protein (AGRP) and neuropeptide Y (NPY) are synthesized in the same neurons in the hypothalamic arcuate nucleus. We have previously shown that NPY/AGRP neurons contain growth hormone (GH) receptor mRNA, and are activated following systemic GH administration. We also reported that NPY inhibits GH secretion when administered centrally. In this study, we have examined the effect of AGRP on GH secretion. Central administration of AGRP (83–132) as a single injection of 1 or 10 μg/rat, or chronic treatment of 1 μg/rat, every 12 h for 7 days, did not alter the GH secretory pattern of adult male rats. AGRP (83–132) at doses of 1–100 nM (4 h) did not alter baseline- and GHRH-induced GH secretion from the rat pituitary cell cultures. These results suggest that AGRP does not play a significant role in the feedback regulation of the GH secretion.

Keywords: Agouti-related protein; Neuropeptide Y; Growth hormone; Hypothalamus; Pituitary; Rat

1. Introduction

Agouti-related protein (AGRP), a 132-amino-acid protein, is the endogenous antagonist for both the melanocortin 3 and 4 receptors [1,2], with its bioactive region contained within the C-terminal 83–132 amino acids [3]. AGRP is a potent orexigenic peptide when administered centrally. The distribution of AGRP mRNA has been examined in mouse and rat brain, and found to be restricted to the hypothalamic arcuate nucleus (ARC), an area known to contain abundant neurons that contain neuropeptide Y (NPY), another potent orexigenic peptide [2,4]. Furthermore, recent work has demonstrated that almost 95% of NPY mRNA-positive neurons also express AGRP mRNA and 98% of AGRP mRNA-positive neurons also express NPY mRNA, suggesting that NPY and AGRP are synthesized in the same neurons in the ARC [5]. We have previously shown that many NPY/AGRP neurons located within the ARC express growth hormone (GH) receptor mRNA [6] and respond to systemic administration of GH by increasing the expression of c-*fos*, a marker for neuronal activity [7,8]. In addition, we and others also have demonstrated that central administration of NPY inhibits the release of GH in rats [9,10], and administration of an anti-NPY serum increases plasma GH levels in 72 h-fasted rats [11], suggesting that NPY has an inhibitory role in the regulation of GH secretion. However, no report has examined the role of AGRP in GH secretion. Therefore, in this study, we have examined the effect of AGRP (83–132) on GH secretion in vivo and in vitro.

2. Materials and methods

2.1. Animal

Adult male Sprague–Dawley rats (380–420 g; Saitama Jikken, Saitama, Japan) were housed in air-conditioned animal quarters with lights on from 0700 to 2100 h. Food and water were provided ad libitum throughout the study.

* Corresponding author. Tel.: +81 3 3822 2131; fax: +81 3 3943 4313.
E-mail address: jkamegai@nms.ac.jp (J. Kamegai).

0167-0115/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2004.08.012

All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Nippon Medical School. The data were analyzed with a one-way analysis of variance followed by Duncan's new multiple range test. A value of $P<0.05$ was interpreted as a significant difference.

2.2. In vivo experiment

Two weeks before the study, rats were anesthetized with ether and a 23-gauge stainless-steel cannula was implanted into right lateral ventricle using a stereotaxic apparatus, as previously described [12].

2.2.1. GH secretion

Five days before the study, rats were provided with an indwelling right atrial cannula under ketamine and xylazine anesthesia for the undisturbed sampling of blood. Serial blood specimens (20 μl) were withdrawn via the indwelling right atrial cannula every 10 min with an automatic blood sampling device as previously described [13]. Blood was collected from 0800 to 2000 h. Each blood specimen was automatically diluted with heparinized saline (1:5) and assayed directly for rat GH. The GH content was measured with a double-antibody radioimmunoassay using materials supplied by NIDDK, NIH (Bethesda, MD, USA). All samples were assayed at the same time. All values are expressed as ng/ml blood in terms of the NIDDK reference preparation, rat GH-RP-2. In the first study, the effects of an acute single intracerebroventricular (icv) administration of AGRP (83–132) (1 or 10 μg/rat; Phoenix Pharmaceuticals, CA, USA) on the blood GH secretory pattern was observed in adult male rats. AGRP (83–132) dissolved in 10 μl of saline or the vehicle was given icv 6 h after the beginning of the blood sampling. In the second study, the effects of chronic icv administration of AGRP (83–132) on the secretory pattern of GH in the blood was examined in adult male rats. AGRP (83–132) (1 μg/rat) or saline was injected every 12 h (at 0900 and 2100 h) for 7 days. Blood was collected from 0800 to 1700 h of the seventh day. To identify GH pulses and baseline levels, data obtained from each rat were processed with the PULSAR computer program [13]. The criteria for identification of GH pulses have been described previously [13]. Values exceeding 150 ng/ml or less than 1.5 ng/ml were replaced by 150 or 1.5 ng/ml, respectively. These represented the ED_{90} and ED_{10} values of the radioimmunoassay for rat GH. The area under the curve depicting the concentration of GH in the blood was measured with a planimeter.

2.2.2. Food intake

To investigate feeding behavior induced by the central administration of AGRP, food intake was measured after AGRP (83–132) (1 and 10 μg/rat) or saline was injected. Immediately after injection, animals were returned to cages containing a known amount of chow. At 4 h after injection, remaining food was weighed.

2.3. In vitro experiment

Anterior pituitaries were enzymatically and mechanically dissociated into single cells and cultured for 3 days before experimental treatment, as previously described [14]. Cells were plated onto 24-well tissue culture plates at 50,000 cells/ml of αMEM supplemented with 10% horse serum. After a 3-day culture period, wells were rinsed with serum-free medium, and then the medium was removed and replaced with 1 ml of medium to which AGRP (83–132) was added to achieve a final concentration of 1, 10, or 100 nM in the presence or absence of 1 nM GH-releasing hormone (GHRH; Sigma, Tokyo, Japan).

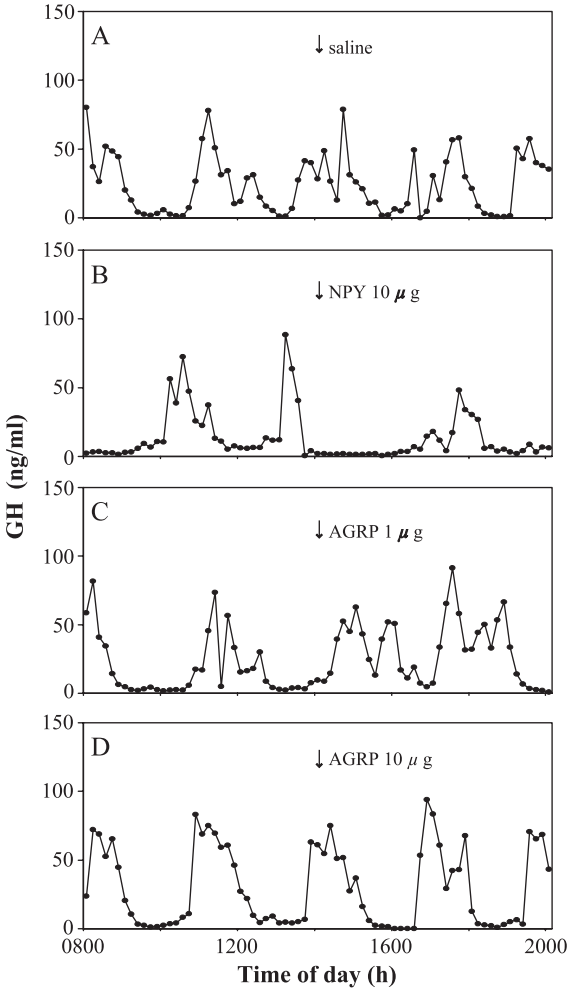

Fig. 1. Effect of single intracerebroventricular (icv) administration of AGRP (83–132) or NPY on blood GH profiles in adult male rats. Individual representative 12-h blood GH profiles in a saline infused rat (A), NPY (10 μg/rat) infused rat (B), an AGRP (83–132) (1 μg/rat) infused rat (C), and an AGRP (83–132) (10 μg/rat) infused rat (D). AGRP (83–132) or NPY was given icv at 1400 h, and blood specimens were obtained every 10 min.