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Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats

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Abstract

Rationale: Ghrelin is a peptide of 28 amino acids found in mammals that increases the release of growth hormone, food intake, and body weight.
Objectives: We investigated the relationship between ghrelin and the states of anxiety and depression by giving rats either antisense DNA for ghrelin, scrambled DNA or vehicle into the lateral ventricle of rats.
Results: In forced swimming tests, rats that received antisense DNA decreased the length of time that they were immobile in the water. Ghrelin antisense oligonucleotides produced an anxiolytic-like effects in the elevated plus maze test, black and white test, or conditioned fear tests. Treatment with antisense DNA for ghrelin significantly decreased rat body weight. No significant effect on general locomotor activity was seen.
Conclusions: These results suggest that administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats.

Keywords: Ghrelin; Antisense-DNA; Antidepressant response; Anxiolytic response; Forced swimming test; Anxiety; Elevated plus maze test; Black and white test; Conditioned fear test

1. Introduction

Growth hormone (GH) that is an anterior pituitary hormone that stimulates protein synthesis and lipolysis by release of fatty acids from adipose tissue. Growth hormone releasing hormone (GHRH) is secreted by the hypothalamus; in the anterior pituitary, it causes release GH through interaction with GHRH receptor (GHRH-R). However, there is another route that stimulates release of GH. GH secretagogue (GHS) stimulates the anterior pituitary to release GH through interaction with the GHS receptor (GHS-R), which is also called an orphan receptor (Howard et al., 1996; Dieguez and Casanueva, 2000).

Ghrelin, which was identified for the first time by Kojima et al. in rat stomach, is a novel peptide of 28 amino acids that acts as endogenous ligand for the GHS-R (Kojima et al., 1999; Kangawa et al., 2004; Kojima and Kangawa, 2005). Thus, one function of ghrelin is to increase pituitary release of GH (Arvat et al., 2000; Date et al., 2000; Wren et al., 2000). Regional distribution of ghrelin receptors suggest that the peptide could be related to emotional processes. For instance, fasting plasma levels of the ghrelin have been found to be elevated in person with anorexia nervosa (Otto et al., 2001; Nakai et al., 2003; Tolle et al., 2003; Ariyasu et al., 2004), and clinical trials of ghrelin have been initiated (Nagaya et al., 2001; Enomoto et al., 2003; Akamizu et al., 2004). In addition, it has been reported that ghrelin stimulates food intake, and consequently, an increase in body weight (Ukkola, 2004; Laferrere et al., 2005; Ueno et al., 2005).

In other studies, researchers reported that both central (e.g., intra-ventricular) and peripheral (e.g., intraperitoneal) administration of ghrelin is a potent inducer of anxiogenic behavior in mice (Asakawa et al., 2001). Similar research indicates that ghrelin induces anxiogenesis in rats (Carlini et al., 2002, 2004). In the current study, we investigated the relationship between level of ghrelin activity and the presence of depression and/or anxiety by administering either antisense DNA or scramble DNA to rats.

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2. Materials and methods

2.1. Animals

Male Wistar rats were obtained from Kyudo Co., Ltd (Fukuoka, Japan). The body weights of rats (7–9 weeks old) of the three group were 316±29.1 (control group), 307±25.8 (scramble group), and 329±35.4 (antisense group) before treatment with antisense DNA for ghrelin. After treatment with antisense DNA for ghrelin, the body weights of rats of the three group were 359±18.5 (control group), 352±10.3 (scramble group), and 306±36.0 (antisense group) (Fig. 1). The body weight of rats was significantly reduced by administration of antisense DNA for ghrelin [$F(2,31)=3.168$, $p=0.001$]. The rats were paired in each cage where they could freely take food and water. Cages were 15-cm high with a floor size of 22 cm×35 cm. The number of rats was 9–11. The room was controlled with a constant light–dark cycle (12-h light/12-h dark cycle with lights on at 18:00 h), temperature (22–23 °C) and humidity (50–60%). Each experiment was conducted between 10:00 and 13:00 h (dark period). Prior to performance of any experiment, rats were handled by research personal for 1 week, 3 weeks, and 3 weeks after their arrival at the facility. All experiments were approved by the Animal Ethics Committee of Oita University Faculty of Medicine.

2.2. Treatments

The rats were divided into three groups. One group was administered antisense DNA for ghrelin into cerebrospinal fluid in order to control the synthesis of ghrelin. Second group was administered scrambled-sense DNA. The base sequence of ghrelin antisense DNA was 5′-AGT-TGC-AGA-GGA-GGC-AGA-AGC-T, whereas the base sequence of the scrambled DNA was 5′-ACC-CGG-GGG-GGA-GTT-ACC-CAG-T. Both forms of DNA were obtained from Sigma-Aldrich Japan (Tokyo, Japan). The control group was administered saline into cerebrospinal fluid.

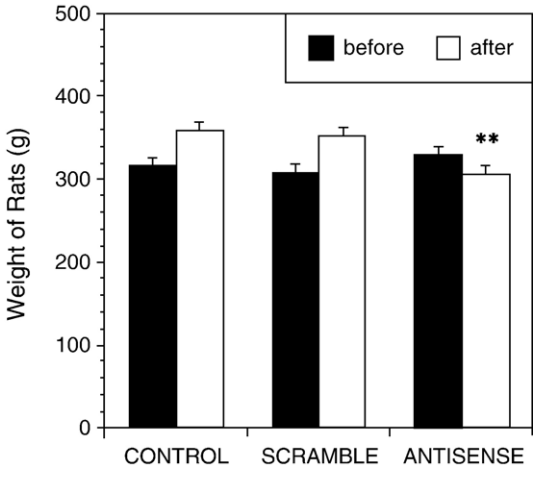

Fig. 1. The body weights of rats. Data are means±SEM. **$p<0.01$ compared to other two groups.

Table 1
Effects of the intracerebroventricular injection of antisense of ghrelin in elevated plus-maze test

	Control	Scramble	Antisense
Number of entry in the open arms	0.75±0.25	1.00±0.36	2.38±0.53**
Number of entry in the closed arms	4.88±0.77	3.50±0.56	3.88±0.99
Total numbers of entry in each arms	5.63±0.65	4.50±0.67	6.25±1.35
Time spent on the open arm	16.33±4.27	24.86±12.67	57.26±12.22**
Time spent on the closed arm	283.67±4.27	275.14±12.67	242.44±12.22**

Data are means±SEM. **$p<0.01$ compared to other two groups.

2.3. Surgical procedures

Each rats was anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally), and underwent stereotactic implantation with a Brain-Infusion Kit (Model 1007D, Alzet Corp., Palo Alto, CA, USA) into the lateral cerebral ventricle (0.92 mm caudal and 1.6 mm lateral to the bregma and 3.5 mm deep); and the Micro-Osmotic Pumps (Model 1003D, Alzet Corp.) were placed into the subcutaneous tissue of the back. After the operation, rats were injected with ceftriaxone sodium (20 mg/kg, intraperitoneally) for prophylaxis of bacterial infection. The recovery time between the surgery and the experiments was 3 days. Each rat received constant infusion of ghrelin antisense oligonucleotides or the scrambled DNA sequence (0.5 μg/0.5 μL/h) into lateral ventricle for 3 days.

2.4. Behavioral testing

On the first day of postoperative DNA infusion, all rats performed the elevated plus-maze test, the black and white test, and electric foot shocks for conditioned fear test. On the second day, rats performed the conditioned fear test and the forced swimming test. All the experiments were approved by the animal ethics committee of Oita University Faculty of Medicine.

2.5. Elevated plus-maze test

The elevated plus-maze test was invented by Lister (1987) to investigate the behaviors related to anxiety. Our apparatus was made of nontransparent plastic and consisted of two opposite open arms (50×10 cm) without side walls and two enclosed arms (50×10 cm) with walls that were 40 cm high. Each arm extended in an overall cross shape from a central platform (10×10 cm). This plus-maze was elevated 50 cm above the floor. Red lights (60 W) were placed 1 m above the maze for lighting. Individual rats were placed on the platform facing on open arm, immediately left alone in the test room, and observed for 5 min with camcorder. Behavioral data were recorded regarding the following five activities: (1) the number of entries into the open arms; (2) the number of entries into the closed arms; (3) the total number of entries of each arm; (4) the percentage of number into open arms; and (5) total time spent in the open arms. An entry into another arm or platform was scored