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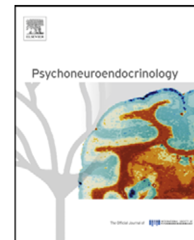

Parathyroid hormone-related protein has an anorexigenic activity via activation of hypothalamic urocortins 2 and 3

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Summary Cancer cachexia is reported to be a major cause of cancer-related death. Since the pathogenesis is not entirely understood, only few effective therapies have been established. Since myriad tumors produce parathyroid hormone-related protein (PTHrP), plasma concentrations of PTHrP are increased in cancer cachexia. We measured the food intake, gastric emptying, conditioned taste aversion (CTA), and gene expression of hypothalamic neuropeptides in mice after administering PTHrP intraperitoneally. We administered PTHrP intravenously in rats and examined the gastroduodenal motility and vagal nerve activities. We also examined whether chronic administration of PTHrP influenced the food intake and body weight. Peripherally administered PTHrP induced negative energy balance by decreasing the food intake and gastric emptying; however, it did not induce CTA. The mechanism involved the activation of hypothalamic urocortins 2 and 3 through vagal afferent pathways and the suppression of gastroduodenal motor activity. The continuous infusion of PTHrP reduced the food intake and body weight gain with a concomitant decrease in the fat and skeletal muscle. Our findings suggest that PTHrP influences the food intake and body weight; therefore, PTHrP can be considered as a therapeutic target for cancer cachexia.
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1. Introduction

Cancer cachexia syndrome is characterized primarily by anorexia, weight loss, poor mental and physical performance, and a compromised quality of life, none of which are resolved by forced nutrient intake (Toomey et al., 1995; Inui, 2002; Harvey et al., 1979). Cachexia is reportedly responsible for up to 30% of cancer-related deaths overall, and 30—50% of deaths in patients with gastrointestinal tract cancers. However, few effective therapies have been established because its pathogenesis is not entirely understood. Among symptoms of cancer cachexia syndrome, weight loss is a key feature of cachexia (Toomey et al., 1995; Inui, 2002; Harvey et al., 1979). In general, patients lose body weight because of reduced food intake. Numerous studies have reported that hypothalamic neuropeptides and gut motility play a pivotal role in the regulation of food intake and the etiology of eating abnormalities (Schwartz et al., 2000; Inui et al., 2004).

Various humoral mediators including parathyroid hormone-related protein (PTHrP), tumor necrosis factor (TNF)α, interleukin (IL) 1 and IL6 are reportedly produced in cancer cachexia syndrome (Toomey et al., 1995; Inui, 2002; Strewler et al., 1987; Moseley et al., 1987; Burtis et al., 1987; Strewler, 2000). Parathyroid hormone-related protein has been identified and its cDNA was cloned from human tumors associated with the syndrome of humoral hypercalcemia of malignancy. Since then, a substantial amount of investigative effort has specifically addressed the role of PTHrP in calcium metabolism and function (Strewler, 2000). Parathyroid hormone-related protein-knockout mice display a systemic chondrodysplasia that is lethal at birth (Karaplis et al., 1994). Whereas PTH is a classic systemic hormone charged with regulating calcium and phosphorous homeostasis, PTHrP acts exclusively in an autocrine or paracrine manner. However, numerous tumors, including colon, lung, renal, breast, skin, prostate, and ovarian carcinomas, and T-cell leukemia produce PTHrP; consequently, plasma concentration of PTHrP is increased in tumor-bearing animals and cancer cachexia syndrome (Burtis et al., 1990; Gaich and Burtis, 1990; Pardo et al., 2004). So far, whether PTHrP is involved in the regulation of food intake, gut motility, and body weight remains unknown. Herein, we demonstrate that PTHrP influences food intake, gut motility, and body weight and may be a therapeutic target for cancer cachexia syndrome.

2. Materials and methods

2.1. Animals and drugs

We used male ddy mice (34—37 g, 8—9 weeks of age; Japan SLC Inc., Shizuoka, Japan), and male Wistar rats (230—280 g, 8—10 weeks of age; CLEA Japan Inc., Tokyo, Japan). The mice and rats were housed individually in a regulated environment (22 $\pm$ 2 °C, 55 $\pm$ 10% humidity, 12:12 h light:dark cycle with light on at 7:00 a.m.). Food and water were available *ad libitum* except as indicated. They were used only once each in the experiment. Our university animal care committee approved all experiments. Mouse/rat $PTHrP_{1-34}$ was purchased from Peptide Institute Inc. (Osaka, Japan). The Yanaihara Institute Inc. (Shizuoka, Japan) produced antibodies against urocortins 1, 2 or 3. Immediately before administration, PTHrP was diluted in physiological saline containing 2% L-cysteine which also served as control solutions. The PTHrP doses were determined on the basis of previous studies (Burtis et al., 1990; Iguchi et al., 2006) and our preliminary experiments on food intake.

2.2. Production of antisera

Production of antisera was carried out against urocortins 1, 2 and 3. Synthetic mouse urocortin 1 (15.0 mg), urocortin 2 (15.0 mg) or urocortin 3 (15.5 mg) and porcine thyroglobulin (Sigma—Aldrich Corp., MO, USA) (54.6 mg) were dissolved in 0.1 M HEPES buffer (6 ml, pH 8.1). To that mixture, dimethyl suberimidate 2HCl (Pierce Chemical Co., IL, USA) (4.4 mg) was added. The mixture was stirred for 2 h at room temperature. The ensuing conjugate (1.5 ml) was emulsified with Freund's complete adjuvant (Calbiochem-Behringer, CA, USA) (1.5 ml) with a mixer for 45 min in an ice bath. The emulsion was injected intradermally into multiple sites of three Japanese white female rabbits. For primary immunization, each rabbit received a portion of the emulsion containing approximately 1.25 mg of peptide. Immunization was performed at 2-week intervals using half the dose of the immunogen used for primary immunization. The rabbits were bled from the ear vein 10 days after each immunizations. After the sixth immunization, one of the three rabbits gave a high titer antiserum. The antisera specificity was characterized by mouse urocortin 1, 2 and 3 specific enzyme immunoassay (EIA) (Supplementary Fig. S1—S3).

2.3. Intracerebroventricular (icv) substance application

For icv administration, the mice were anesthetized with sodium pentobarbital (80—85 mg/kg intraperitoneal (i.p.) administration) and placed in a stereotaxic instrument 7 days before experiments. A hole was made in each mouse's skull using a needle inserted 0.9 mm lateral to the central suture and 0.9 mm posterior to the bregma. A 24-gauge cannula beveled at one end over a distance of 3 mm was implanted into the third cerebral ventricle for icv administration. The cannula was fixed to the skull with dental cement and capped with silicon without an obtruder. A 27-gauge administration insert was attached to a microsyringe using PE-20 tubing.

2.4. Feeding tests

Before feeding tests, mice were deprived of food for 16 h with free access to water, or were given free access to food and water. A standard diet (CLEA Japan Inc., Tokyo, Japan) was used except for the experiment testing the effect of vagotomy on feeding suppression induced by PTHrP, which used a liquid diet (Oriental Yeast Co. Ltd., Tokyo, Japan). Dark-phase feeding studies administrations were done immediately before lights-off (7:00 p.m.) for non-food-deprived mice. For food-deprived mice, PTHrP was administered at 10:00 a.m. Food intake was measured by subtracting uneaten food from initially premeasured food at 20 min, 1 h, 2 h, and 4 h after administration and checking the food spillage.

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