to the amount of sodium channel blocking, including $Na_v1.2$ and $Na_v1.8$.[40] These recent reports support that suppression of $Na_v1.2$ function by APAS might be a mechanism underlying the analgesic effects of allopregnanolone.

In conclusion, APAS and PAS have diverse effects on $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$ α subunits expressed in *Xenopus* oocytes, with differences in the effects on sodium channel gating. In particular, only APAS inhibited sodium currents of $Na_v1.2$ at pharmacologically relevant concentrations. These results raise the possibility that suppression of $Na_v1.2$ by APAS may be important for pain relief by allopregnanolone and provide a better understanding of the mechanisms underlying the analgesic effects of allopregnanolone. However, further studies are needed to clarify the relevance of sodium channel inhibition by APAS.

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Competing Interests

The authors declare no competing interests.

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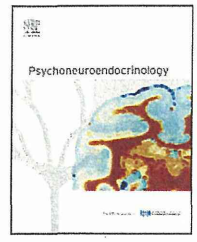

Parathyroid hormone-related protein has an anorexigenic activity via activation of hypothalamic urocortins 2 and 3

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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 ± 2 °C, 55 ± 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.

2.5. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Mice were deprived of food for 16 h with free access to water; then they were treated with PTHrP (300 pmol/mouse) or vehicle every 6 h for 12 h, with the third and final administration at 30 min before the mice were killed by cervical dislocation. Immediately after, the hypothalamic block, stomach and epididymal fat were removed, frozen on dry ice, and stored at −80 °C until preparation of real-time RT-PCR. Using the RNeasy Mini Kit (Qiagen Inc., Tokyo, Japan) RNA was isolated from the hypothalamic block, stomach and epididymal fat. Quantification of mRNA levels was performed with SYBR-green chemistry (Qiagen Inc., Tokyo, Japan) using a one-step RT-PCR reaction on a sequence detection system (ABI PRISM 7700; Applied Biosystems Japan, Tokyo, Japan). The reaction was performed under standard conditions recommended by the manufacturer. We used the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as an internal control. All expression data were normalized to GAPDH expression level from the same individual sample. The following primers were used for real-time RT-PCR: GAPDH forward, ATGGTGAAGGTCGGTGTGAA; and reverse, GAGTGGAGTCATACTGGAAC. Neuropeptide Y (NPY) forward, TTTCCAAGTTTCCACCCTCATC; and reverse, AGTGGTGGCATGCATTGGT. Agouti-related protein (AGRP) forward, GAGTTCCCAGGTCTAAGTCTGAATG; and reverse, ATCTAGCACCTCCGCCAAAG. Orexin A forward, CGTAACTACCACCGCTTTAGCA; and reverse, TGCCATTTACCAAGAGACTGACAG. Melanin-concentrating hormone (MCH) forward, GGAAGATACTGCAGAAAGATCCG; and reverse, ATGAAACCGCTCTCGTCGTT. Cocaine and amphetamine-regulated transcript (CART) forward, GCAGATCGAAGCGTTGCAA; and reverse, TTGGCCGTACTTCTTCTCGTAGA. Proopiomelanocortin (POMC) forward, GGCTTGCAAACTCGACCTCT; and reverse, TGACCCATGACGTACTTCCG. Corticotropin-releasing factor (CRF) forward, CGCAGCCCTTGAATTTCTTG; and reverse, TCTGTTGAGATTCCCCAGGC. Urocortin 1 forward, ACTGTCCATCGACCTCACCTTC; and reverse, AAGGCTTTCGTGACCCCATA. Urocortin 2 forward, CCTCAGAGAGCTCCTCAGGTACC; and reverse, GGTAAGGGCTGGCTTTAGAGTTG. Urocortin 3 forward, CGCACCTCCAGATCAAAAGAA; and reverse, GGGTGCTCCCAGCTCCAT. Ghrelin forward, AGCATGCTCTGGATGGACATG; and reverse, GCAGTTTAGCTGGTGGCTTCTT. Leptin forward, CTGTGGCTTTGGTCCTATCT; and reverse, TGATAGACTGCCAGAGTCTG. Adiponectin forward, TGCTACTGTTGCAAGCTCTC; and reverse, AGGACCAAGAAGACCTGCAT.

2.6. Immunohistochemistry

Mice were deprived of food for 16 h with free access to water, then administered intraperitoneally with PTHrP (300 pmol/mouse) or vehicle. The mice were anesthetized with sodium pentobarbital (80–85 mg/kg i.p.) and perfused with 4% paraformaldehyde, 0.5% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer 90 min after administration. Brains were removed and postfixed with 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer. The brains were cut into 20-μm-thick coronal sections in a cryostat. Immunohistochemistry for c-fos protein expression was performed using ABC and DAB methods, whereas immunohistochemistry for urocortins 1, 2 and 3 was performed using immunofluorescence methods according to previous studies (Chen et al., 2005). Positive reaction was observed under light microscopy (Olympus DX51; Olympus Optical Co. Ltd., Tokyo, Japan) or laser scanning microscope (LSM 510; Carl Zeiss Inc. Japan, Tokyo, Japan).

2.7. Gastroduodenal motility

Gastroduodenal motility was measured in conscious, freely moving rats by manometric method. Rats were deprived of food and given free access to water for 16 h before the abdominal operation. They were anesthetized with pentobarbital sodium (50 mg/kg i.p.); then a motility recording device was implanted as follows. Two manometric catheters (3-Fr, 1 mm diameter; Atom Medical Corp., Tokyo, Japan) with side holes were inserted through the fistula at the gastric body and the tips were placed at the gastric antrum and 3 cm distal to the pylorus. Catheters were fixed at the gastric wall by purse-string suture and run subcutaneously to emerge at the crown of the neck and were secured at the animals' skin. A catheter (3-Fr, 1 mm diameter) was placed in the right jugular vein, run subcutaneously to emerge at the crown with the manometric catheter, and used for intravenous (i.v.) administration. The catheter was filled with heparinized saline to prevent obstruction. After 1 week, animals were fasted for 16 h before the experiment. On the day of the experiment, a manometric catheter was connected to a pressure transducer (TP-400T; Nihon Kohden Corp., Tokyo, Japan), and connected to the infusion swivel (dual type, 20-gauge; Instech Laboratories Inc., PA, USA) to allow free movement. The catheter was infused continuously with bubble-free 0.9% saline at a rate of 1.5 ml/h by a low-compliance capillary infusion system using a heavy-duty pump (CVF-3100; Nihon Kohden Corp.). The PTHrP (300 pmol/rat) was dissolved in physiological saline containing 2% L-cysteine in a 300 μl volume for i.v. administration.

2.8. Gastric emptying

Before experiments for gastric emptying, mice were deprived of food for 16 h with free access to water. The fasted mice had free access to pre-weighed pellets for 1 h; they were then administered i.p. with PTHrP (3–300 pmol/mouse) or vehicle. The mice were deprived of food again for 2 h after administration. Food intake was measured by weighing uneaten pellets. Mice were killed by cervical dislocation 3 h after the start of experiments. Immediately after, the stomach was exposed by laparotomy, quickly ligated at both the pylorus and cardia, then removed; then the dry content was weighed. Contents were dried using a vacuum freeze-drying system (Model 77400; Labconco Corp., MO, USA). Gastric emptying was calculated according to the following formula: gastric emptying (%) = {1 − (dry weight of food recovered from the stomach/weight of food intake)} × 100.

2.9. Truncal vagotomy

Four days before experiments, truncal vagotomy was performed. The mice were anesthetized with sodium pentobar-

bital (80–85 mg/kg i.p.). After a midline incision of the abdominal wall, the stomach was covered with sterile gauze moistened with warm saline. The lower part of the esophagus was exposed and the anterior and posterior branches of the vagal nerve were incised. At the end of the operation, the abdominal wall was sutured in two layers. In sham-operated mice, vagal trunks were similarly exposed, but not cut. Vagotomized and sham-operated mice were maintained on a nutritionally complete liquid diet. Completeness of vagotomy was verified during postmortem inspection. Mice were fixed for enzyme histochemistry. Loss of acetylcholine esterase positive fibers in the gastrointestinal tracts was determined by light microscopic observation.

2.10. Electrophysiological study

Electrophysiological measurements were performed to record changes in activity of gastric and hepatic vagal nerves. Rats were anesthetized with urethane (1 g/kg i.p.), and a tracheal cannula was inserted. Under a dissecting microscope, a nerve filament was dissected from the peripheral cut end of the vagus nerve to record afferent nerve activity via a pair of silver wire electrodes. A rate meter with a rest time of 5 s was used to observe the time course of nerve activity. Administration of PTHrP (0.1–10 μg/rat) was done through a small catheter inserted into the inferior vena cava in a 100 μl volume. The effect of PTHrP on the vagal nerve activity was determined by comparing the mean number of impulses per 5 s over 50 s before and after the administration.

2.11. Conditioned taste aversion

A standard two-bottle taste aversion paradigm was used for conditioned taste aversion (CTA) assay. Mice were accustomed to having access to water for 1 h (10:00–11:00 a.m.) per day for 5 days. On the sixth day, mice received an i.p. administration of PTHrP (300 pmol/mouse) or vehicle following access to a 0.5% (v/v) sodium saccharin solution instead of water. Intraperitoneal administration of

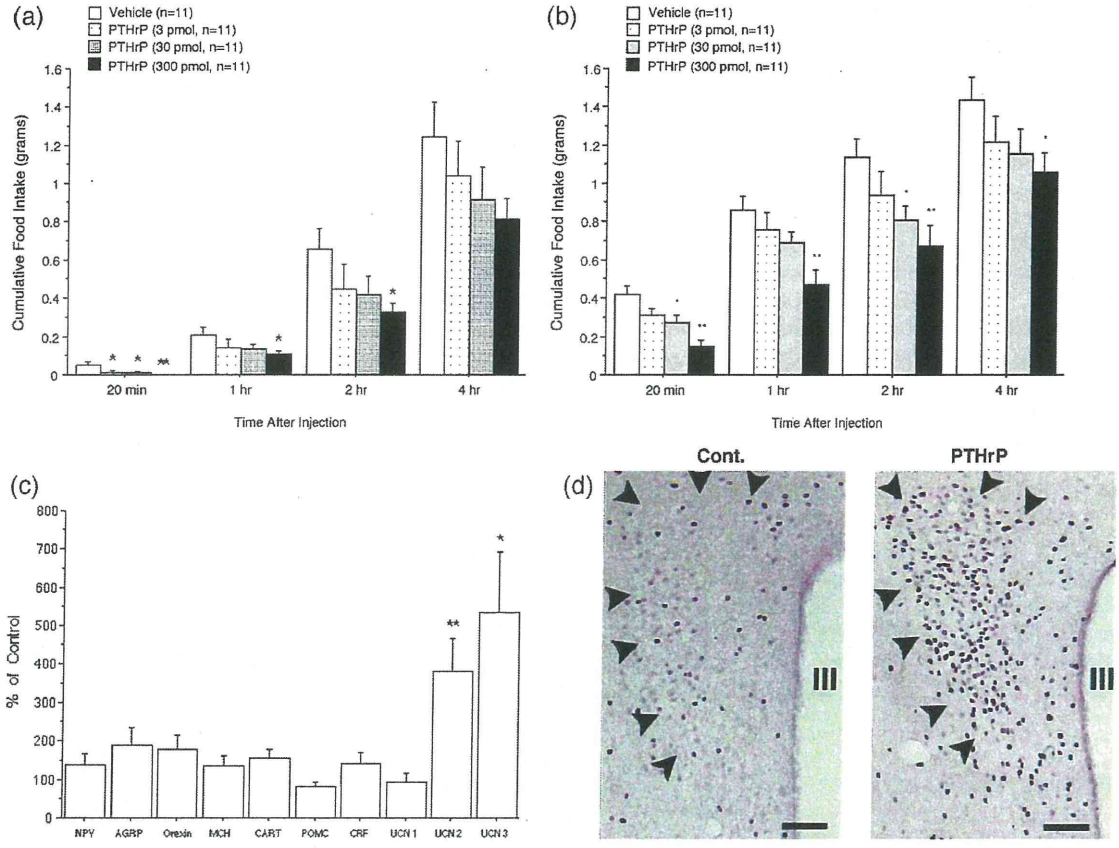

Figure 1 PTHrP has an anorexigenic activity. (A and B) Effects of intraperitoneally administered PTHrP (3–300 pmol/mouse) on cumulative food intake in non-food-deprived mice (A) and food-deprived mice (B). (C) Effects of intraperitoneally administered PTHrP (300 pmol/mouse every 6 h for 12 h) on hypothalamic peptides mRNA levels, as assessed by real-time RT-PCR in food-deprived mice, expressed as a percentage of vehicle treated control ($n = 5–6$). (D) Effects of intraperitoneally administered PTHrP (300 pmol/mouse) on c-Fos expression in the paraventricular nucleus 90 min after administration ($n = 3–4$). Scale bars = 50 μm. (E) Photomicrographs of immunohistochemical demonstration for urocortins 1, 2 and 3 in PVN ($n = 3–4$). Scale bars = 50 μm. (F) Antagonistic effects of either anti-mouse urocortin 2 or 3 antiserum administered icv (4 μl/mouse) on feeding induced by i.p. administration of PTHrP (300 pmol/mouse) in food-deprived mice. Each bar represents the mean ± S.E. n indicates the number of mice used. $^{*}P < 0.05$; $^{**}P < 0.01$ by Bonferroni's t-test.

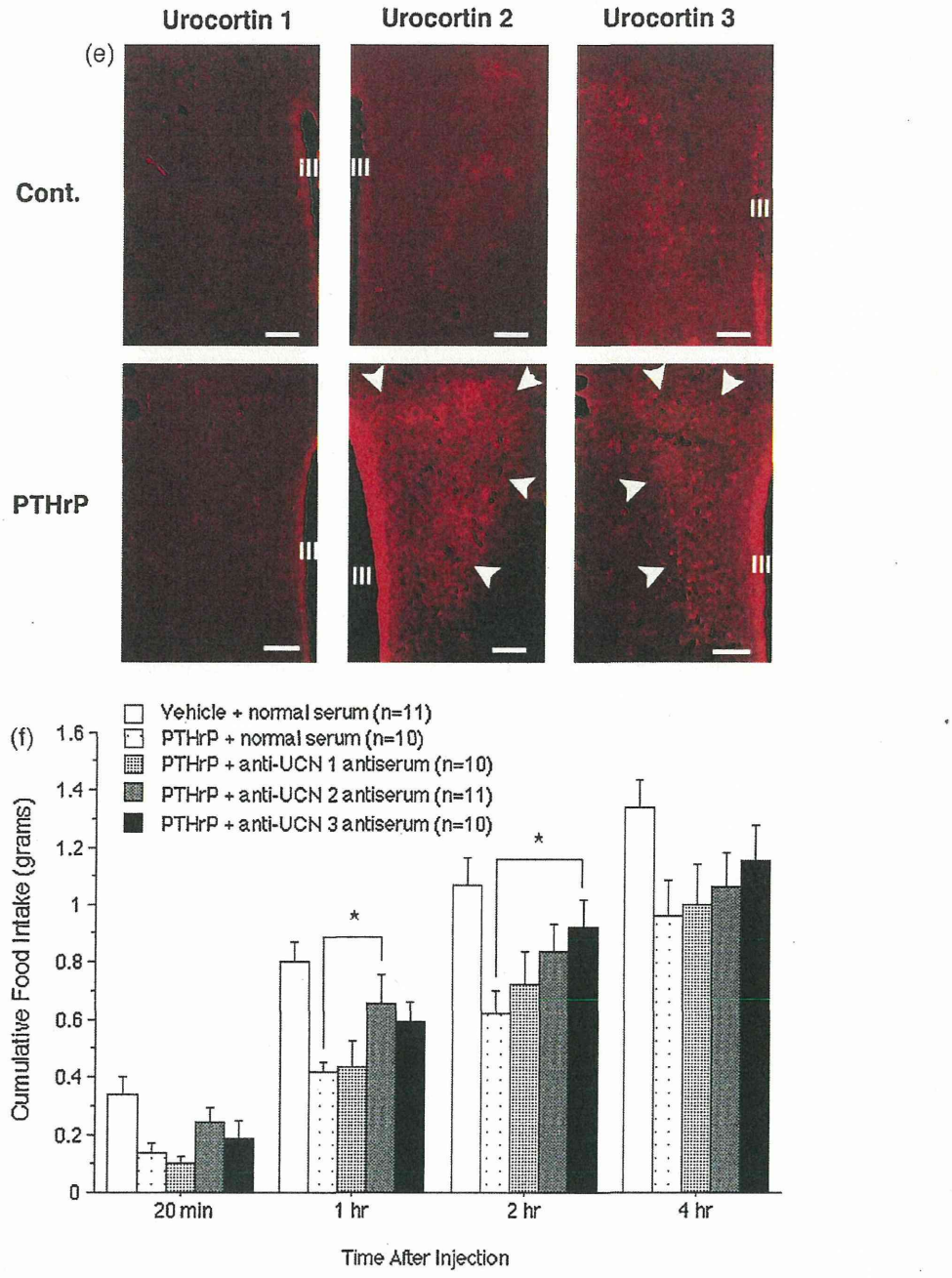

Figure 1. (*Continued*).

LiCl (2% BW of a 0.15 M solution) or physiological saline served respectively as positive and negative controls. Three days later, mice were offered both water and saccharin solution in separate bottles during their 1-h drinking period. The saccharin preference ratio was calculated according to the following formula: preference ratio (%) = (saccharin intake/total fluid intake) × 100.

2.12. Continuous infusion

Continuous systemic administration of PTHrP was achieved via osmotic minipumps (Alza Corp., CA, USA). Model 1007 mini-osmotic pumps were filled with a 200 μl volume of control solution or PTHrP (12.5 pmol/h). Mice were anesthetized with sodium pentobarbital (80–85 mg/kg i.p.). The pump was implanted i.p. under sterile conditions after a small midline incision according to the manufacturer's instructions. The abdominal wall was sutured in two layers. The actual mean pumping rate, reservoir fill volume, and duration of the pump are 0.5 μl/h, 100 μl, and 7 days, respectively. Food and water intake and body weight were measured daily. On the final day, serum was separated from blood obtained from the orbital sinus under ether anesthesia at the end of the experiment (6 h after removal of food). The entire sampling procedure was done in less than 2 min. Mice were killed by cervical dislocation. Immediately after, the

epididymal fat pad mass, assessed as white adipose tissue (WAT), and the gastrocnemius muscle were removed and weighed. Blood glucose and serum triglycerides were measured respectively using the glucose oxidase method and enzymatic method (Wako Pure Chemical Industries Ltd., Tokyo, Japan).

2.13. Statistical analysis

Analysis of variance followed by Bonferroni's *t*-test was used to assess differences among groups. Results are expressed as mean value ± S.E. $P < 0.05$ was considered to be statistically significant.

3. Results

PTHrP has an anorexigenic activity. We first examined the effects of i.p. administration of PTHrP on feeding in non-food-deprived mice and food-deprived mice to investigate whether or not PTHrP influences feeding behavior. Peripherally administered PTHrP significantly produced inhibitory effects on feeding behavior in a dose-related manner (Fig. 1A and B). We examined gene expression of hypothalamic neuropeptides in food-deprived mice after i.p. administration of PTHrP to evaluate the possibility that PTHrP acts through the hypothalamic pathway. Real-time RT-PCR analysis showed that PTHrP significantly increased expression of urocortins 2 and 3 respectively by 281% and 436% compared with controls (Fig. 1C). We next examined the effects of i.p. administration of PTHrP on c-fos expression in the hypothalamus. Peripheral administration of PTHrP showed an increase in c-fos expression in the paraventricular nucleus (PVN) (56.2 ± 7.59 vs. 23.3 ± 2.30 number/section [control], $P < 0.02$) (Fig. 1D). Immunohistochemical studies for urocortins 1, 2 and 3 showed that PTHrP increased the intensity of immunoreaction for urocortins 2 and 3 in the PVN compared with controls (Fig. 1E). We also observed that the feeding-inhibitory effect of PTHrP was significantly blocked by icv administration of anti-urocortin 2 or 3 antiserum (Fig. 1F). In addition, we investigated whether or not administered PTHrP influences gene expression of ghrelin and leptin in stomach, and adiponectin, leptin and resistin in WAT of food-deprived mice ($n = 7–8$). PTHrP had no significant effects on gene expression of ghrelin (104.0 ± 18.7% of control) and leptin (124.2 ± 51.9% of control) in stomach, and adiponectin (67.5 ± 9.3% of control) and leptin (130.5 ± 34.5% of control) in WAT.

PTHrP influences gastric emptying and vagal nerve. Next, we examined whether or not PTHrP influences gastroduodenal motility. Intravenous administration of PTHrP disrupted the fasted motor activity in the antrum and duodenum in food-deprived rats (Fig. 2A). In addition, peripherally administered PTHrP decreased the gastric emptying rate in a dose-related manner (Fig. 2B). We also used mice that had received truncal vagotomy to investigate whether or not the feeding-inhibitory effect of PTHrP is associated with a vagally mediated pathway. Although vagotomy is an invasive operation, vagotomy eliminated inhibitory effects on feeding induced by i.p. administration of PTHrP (Fig. 2C). The electrophysiological study showed that i.v. administration of PTHrP significantly increased afferent activity of the gastric and hepatic vagal nerves (Fig. 2D and E). The possibility that the anorectic effect of PTHrP is partly or completely attribute to malaise or nonspecific toxic effects was also investigated. Administration of PTHrP at a dose that reduced food intake did not result in a CTA, whereas lithium chloride produced a robust CTA (Fig. 2F). We also investigated the chronic effects of PTHrP on feeding and body weight. A chronic i.p. infusion of PTHrP for 7 days using an osmotic minipump decreased food intake and body weight gain during the infusion period (Table 1 and Fig. 2G). Fat pad mass and skeletal muscle mass were also decreased by PTHrP administration.

4. Discussion

Here, we show that PTHrP regulates feeding behavior via the hypothalamus and vagal nerves. Since the discovery of leptin and ghrelin, much progress has been made in the study of controlling energy homeostasis (Inui et al., 2004; Flier and Maratos-Flier, 1998; Zhang et al., 2005). Until now, hypothalamic neuropeptides, including NPY, AGRP, orexin, MCH, α-melanocyte stimulating hormone (α-MSH), CART, CRF and urocortin, have been shown to be involved in the regulation of food intake (Schwartz et al., 2000; Inui et al., 2004). Urocortin was identified in 1995 as the second ligand for CRF receptor (Vaughan et al., 1995). Whereas CRF is mainly involved in the regulation of stress-related behavior and colonic motility, urocortin is involved mainly in the regulation of feeding behavior and gastric motility (Dautzenberg and Hauger, 2002). Previous studies have shown that both urocortins 2 and 3 potently suppress food intake and delay gastric emptying (Hsu and Hsueh, 2001; Ohata and Shibasaki, 2004; Czimmer et al., 2006). Our results support a mechanism of feeding-inhibitory effect of PTHrP involved in urocortins 2 and 3 in the hypothalamus. Feeding stimulatory peptides such as NPY and ghrelin induce the fasted motor activity in the gastrointestinal tracts, whereas feeding-inhibitory peptides such as urocortin, bombesin and cholecystokinin (CCK) disrupt the fasted motor activity in the gastrointestinal tracts (Fujino et al., 2003). In addition, considerable evidence indicates cumulatively that delayed gastric emptying is closely related to anorexia and cachexia, as rapid gastric emptying is to overeating and obesity (Inui, 2002; Duggan and Booth, 1986). In our study, peripherally administered PTHrP disrupted the fasted motor activity in the stomach and duodenum, and decreased the gastric emptying rate. Moreover, our results in vagotomy study show that anorexigenic effects of PTHrP are mediated, at least in part, by the vagal nerve pathway. Electrophysiologic study results also supports vagal mediation of the anorexigenic activity. Numerous studies have shown that the neural mechanism, supporting and extending the hepatic receptor hypothesis, is important for integrating autonomic regulation of gastrointestinal functions as well as feeding behavior (Geary et al., 1993; Niijima and Meguid, 1995). This study indicates the existence of the peripheral receptor mechanism of PTHrP in hepatic vagal afferents. In addition, the effect of PTHrP on the afferent activity of the gastric vagal nerve is similar to that of anorexigenic peptides CCK and peptide YY $(PYY)_{3–36}$, and contrary to that of orexigenic peptide ghrelin (Asakawa et al., 2001; Koda et al., 2005). Behavioral specificity was

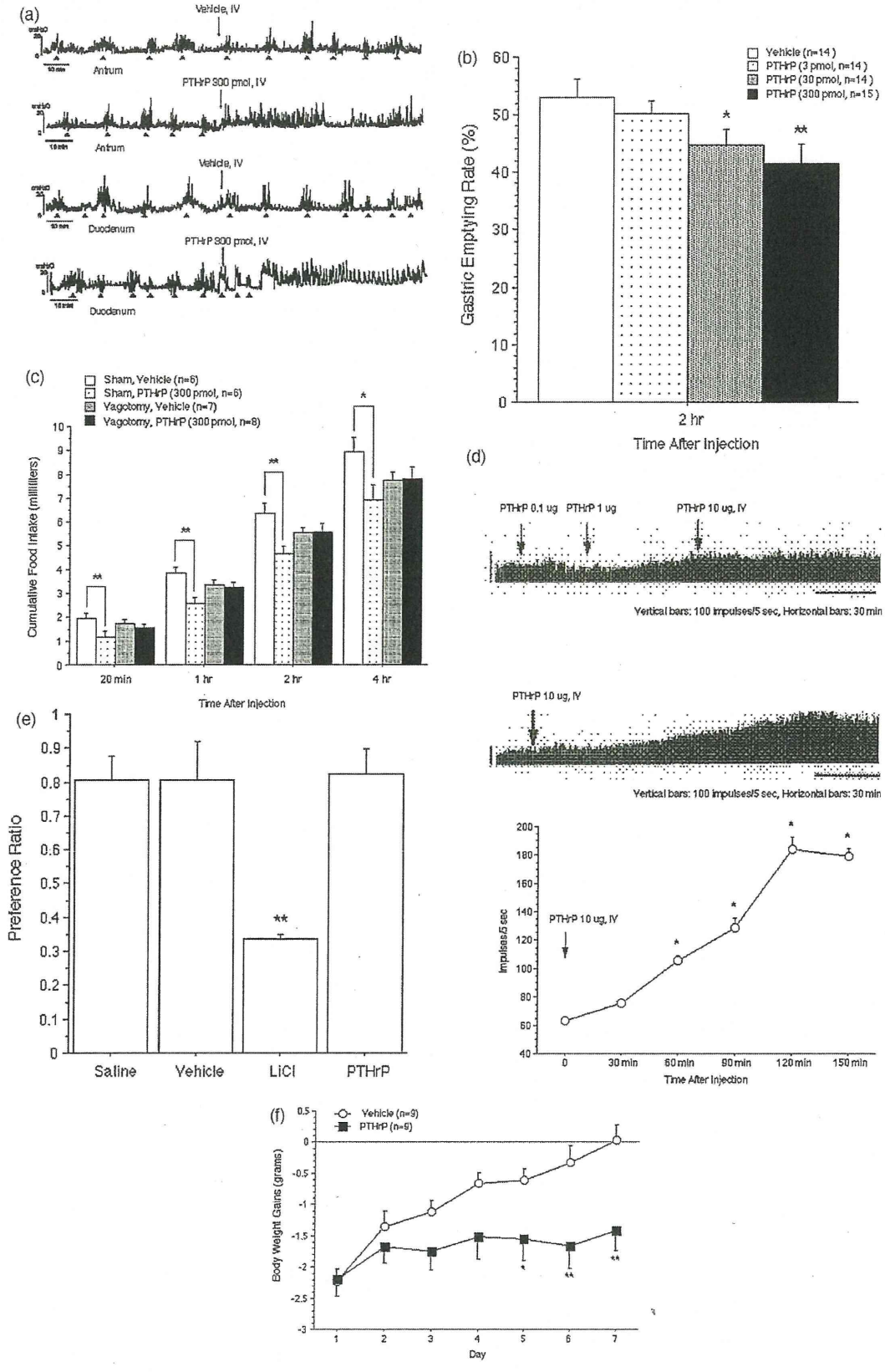

Figure 2 PTHrP influences gastric emptying and vagal nerve. (A) Effects of intravenously administered PTHrP (300 pmol/rat) on the fasted motor activity of the antrum and duodenum ($n = 5–6$). (B) Inhibitory effects of intraperitoneally administered PTHrP (3–300 pmol/mouse) on the gastric emptying rate 2 h after administration. (C) Loss of feeding-inhibitory effect of intraperitoneally

Table 1 Effects of chronic administration of PTHrP on food intake, water intake, epididymal fat mass, gastrocnemius muscle and concentrations of blood glucose, and triglycerides. Results are expressed as mean ± S.E. *n* indicates the number of mice used.

	Vehicle ($n = 9$)	PTHrP ($n = 9$)
Food intake (g/day)	4.007 ± 0.075	3.716 ± 0.100*
Water in take (ml/day)	8.050 ± 0.236	7.874 ± 0.249
Fat pad mass (g)	0.467 ± 0.029	0.362 ± 0.038*
Skeletal muscle (g)	0.344 ± 0.010	0.311 ± 0.011*
Glucose (mg/dl)	145.7 ± 2.819	142.2 ± 5.666
Triglycerides (mg/dl)	103.3 ± 14.88	96.89 ± 9.119

* $P < 0.05$ by Bonferroni's *t*-test.

determined using a CTA assay where the drug administration is paired with ingestion of a palatable substance (saccharin) (Bernstein et al., 1983). Peripheral administration of PTHrP does not induce a CTA, indicating that the anorectic effect of PTHrP is specific and is not caused by aversion. These observations indicate that PTHrP has an anorexigenic activity with its mechanism of action involving the activation of hypothalamic urocortins 2 and 3 through vagal afferent pathways and the suppression of gastroduodenal motor activity.

The most common manifestation of advanced malignant diseases is the development of anorexia and body weight loss. Plasma PTHrP levels are reportedly increased in the majority of advanced cancers, especially cancer cachexia syndrome, and are reduced after chemotherapeutic or surgical treatment (Burtis et al., 1990; Gaich and Burtis, 1990; Pardo et al., 2004). The plasma PTHrP concentrations after IP administration of the effective minimum dose of PTHrP (3 pmol) appear to be very high compared to those found in healthy humans and are similar to those found in animal cancer models and human cancer patients (Burtis et al., 1990; Iguchi et al., 2006). In our study, chronic administration of PTHrP reduced food intake and body weight gain with a concomitant decrease in fat and skeletal muscle. These observations indicate that PTHrP may be involved in the etiology of cancer cachexia syndrome. The degree of cachexia is inversely correlated with the survival time of the patient: it always implies a poor prognosis (Toomey et al., 1995; Inui, 2002; Harvey et al., 1979). Despite numerous efforts devoted to treatment of cancer cachexia, no effective therapy has been established. Recently, Sato et al. have reported that in tumor-bearing rats, the anti-PTHrP antibody ameliorated cancer anorexia and produced a marked recovery of body weight loss (Sato et al., 2003; Onuma et al., 2005). Various combined approaches have been widely adopted for cancer management. Nevertheless, most therapies including anticancer drugs have more or fewer adverse side effects such as anorexia and nausea. Moreover, cachexia not only worsens a patient's general condition; it also compels abandonment of positive therapies that engender unfavorable side effects (Harvey et al., 1979; Schnell, 2003). On the other hand, antibody therapy is characterized by its low incidence of side effects and high target-specificity (Forero and Lobuglio, 2003). Therefore, anti-PTHrP therapy might represent a novel treatment strategy for cancer-cachexia syndrome and various cancers treated with combined anticancer therapies. The development of effective anti-PTHrP antibodies as potential therapeutic agents for cancer cachexia syndrome is awaited with great interest.

In conclusion, this study indicates that PTHrP exhibits a novel, anorexigenic activity. Its mechanism of action involves the activation of hypothalamic urocortins 2 and 3 through vagal afferent pathways and the suppression of gastroduodenal motor activity. Further, PTHrP may play an important role in the etiology of the cancer cachexia syndrome.

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Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psyneuen.2010.02.003.

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administered PTHrP (300 pmol/mouse) after vagotomy in food-deprived mice. (D and E) Effects of PTHrP administered into the inferior vena cava on afferent activity of the gastric vagal nerve (D) and hepatic vagal nerve (E) in rats ($n = 5$). (F) Conditioned taste aversion assay ($n = 6$–7). (G) Effects of chronic administration of PTHrP on body weight gain. Each bar represents the mean ± S.E. *n* indicates the number of mice used. *$P < 0.05$; **$P < 0.01$ by Bonferroni's *t*-test.