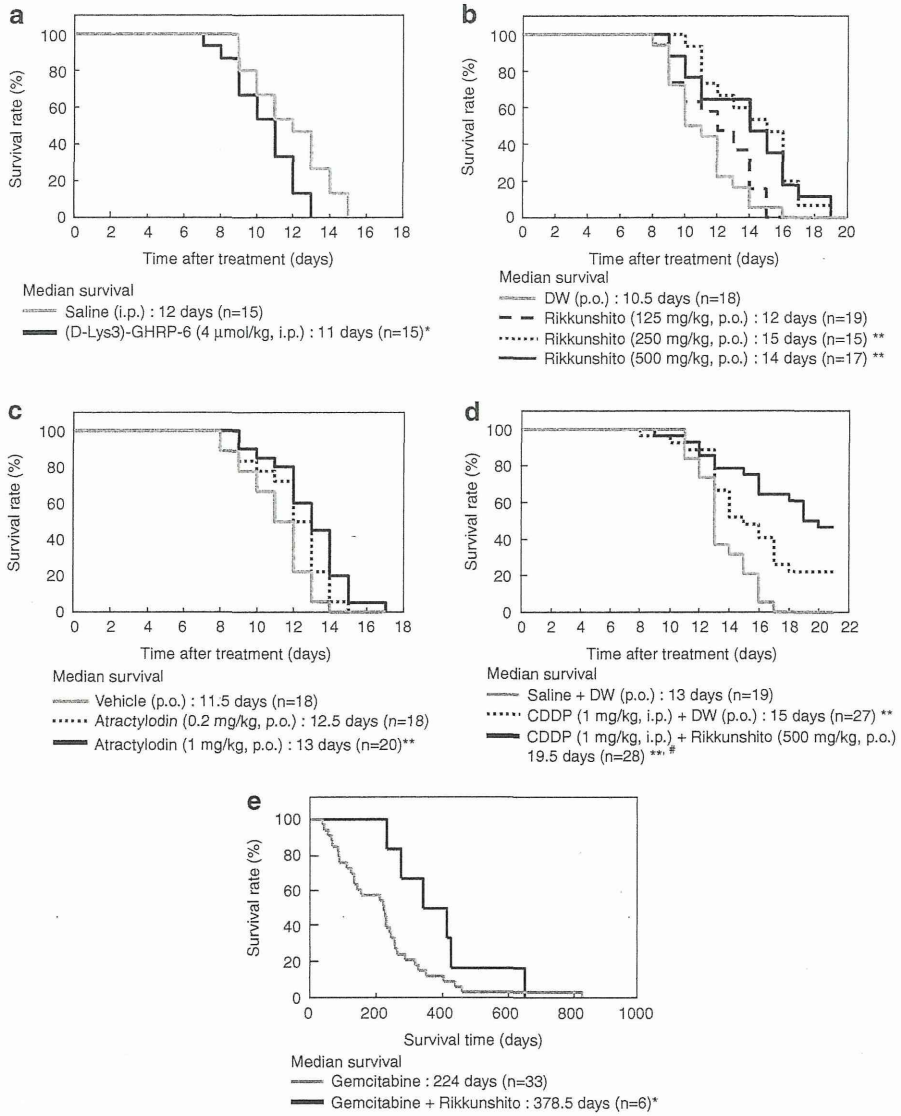

Figure 4 Survival of tumor-bearing rats and patients with pancreatic cancer. (**a**) Administration of (D-Lys3)-GHRP-6 decreased the median survival of tumor-bearing rats. (**b**, **c**) Administration of rikkunshito and atractylodin increased the median survival of tumor-bearing rats. (**d**) Survival of tumor-bearing rats was increased by intraperitoneal (i.p.) administration of cisplatin (CDDP) and further prolonged by co-administration of rikkunshito. (**e**) Rikkunshito prolonged the median survival of pancreatic cancer patients with ascites who were treated with gemcitabine. $^{*}P<0.05$; $^{**}P<0.01$ vs control group; $^{\#}P<0.05$ vs CDDP + DW. DW: distilled water.

inhibitors such as fenfluramine and 5-HT2cR agonists attenuate food intake and weight gain in rodents and humans,[40–42] with the involvement of potentiated MC signaling and decreased ghrelin secretion. 5-HT also inhibits NPY/agouti-related peptide neurons by activating the 5-HT1bR, leading to decreased orexigenic signaling and an inhibitory drive onto POMC cells. However, our previous study suggested that the 5-HT2cR has a major role in the regulation of physiological fasted and fed motor activities in addition to feeding through changes in endogenous ghrelin.[15] In this study, we found that the decreases in food intake and GI motor activities in tumor-bearing rats were recovered after administration of either a 5-HT2cR antagonist or ghrelin. The 5-HT concentration in the hypothalamus is increased in humans and animals with cancer;[43,44] in addition, NPY and dopamine concentrations decrease simultaneously, while 5-HT concentration increases in the PVN at the onset of anorexia in tumor-bearing rats.[45] These findings suggest that 5-HT2cR activation in tumor-bearing rats induces anorexia in part via decreased ghrelin secretion.

We have previously shown that a central 5-HT2cR pathway regulates ghrelin secretion without downstream activation of melanocortin 3/4 receptors.[15] The 5-HT2cR is expressed in many brain regions and its expression is restricted to the central nervous system.[46] Dual-neurohistochemical labeling has revealed that approximately one-half of PVN CRF-containing neurons co-express 5-HT2cR mRNA.[47] In this study, we found that 5-HT activated single CRF neurons isolated from the PVN, and the activities of the CRF neurons were blocked by simultaneous administration of rikkunshito.

Moreover, intracerebroventricular administration of CRF decreased plasma acyl ghrelin in fasted rats. These findings suggest that CRF neurons are involved in 5-HT-regulated ghrelin secretion. Hypothalamic 5-HT and CRF activities are stimulated by proinflammatory cytokines in the circulation and the hypothalamus.[13,48] Here we demonstrate that a CRF receptor antagonist improved cancer anorexia–cachexia, and that administration of the 5-HT2cR antagonist or rikkunshito reduced hypothalamic CRF levels and anxiety-related behaviors in tumor-bearing rats. The improvement in anxiety by rikkunshito may lead to a higher quality of life in cancer patients. Some studies suggest that ghrelin induce anxiogenesis, whereas others suggest that the elevated ghrelin helps animals cope with stress by producing anxiolytic-like response.[49] Future studies are needed to sort out the effect of ghrelin on anxiety-like behavior as in the case for NPY.[50] Importantly, our findings demonstrate that a hypothalamic 5-HT-CRF receptor pathway that regulates ghrelin secretion has a major role in cancer anorexia–cachexia.

The GHS-R is reportedly expressed in vagal afferent neurons, and the gastric vagus nerve system is involved in the effect of ghrelin on food intake and GI motor activities.[51,52] We demonstrated that ghrelin decreased the afferent activity of the gastric vagus nerve. Gastric ghrelin signaling via vagal afferents stimulated the efferent activities of both the gastric and the celiac branches of the vagus nerve and suppressed the activity of the sympathetic nerve. Peripheral administration of a higher dose of ghrelin increased the discharge rate of the vagal efferent nerve, probably in part through the GHS-R in the ARC of the hypothalamus. We also showed that rikkunshito activated the efferent vagus nerve, which may be mediated by both the vagal afferent nerve and the direct central action (Figure 5). In addition, we found that ghrelin-induced cellular signaling in GHS-R-expressing cells was enhanced by pretreatment with rikkunshito and its active components, such as atractylodin, which stimulate ghrelin/GHS-R binding activity. Similar potentiating effects of rikkunshito were observed in rat ARC NPY neurons. These findings suggest that the physiological functions of endogenous ghrelin are enhanced by the dual actions of rikkunshito, which involve the stimulation of ghrelin secretion and the activation of GHS-R activity, possibly due to allosteric changes in the receptor. This potentiation of the ghrelin effect by rikkunshito on NPY neurons could be orexigenic because the activity of ghrelin-responsive NPY neurons is coupled to feeding.[19,53]

Our study indicated the adverse effect of (D-Lys3)-GHRP-6 on survival in tumor-bearing rats, suggesting that the potentiation of ghrelin signaling is critical to the attenuation of anorexia–cachexia and the prolongation of survival in subjects with cancer. Rikkunshito and its active component, atractylodin, prolonged survival in these animals, and this effect was enhanced by the concomitant administration of CDDP. Cancer patients receiving chemotherapy or radiation therapy may experience nausea, vomiting, taste changes, stomatitis and diarrhea, which could contribute to weight loss and decreased survival. Therefore, cancer anorexia–cachexia syndrome is a major obstacle in cancer chemotherapy.[6] Rikkunshito was recently reported to suppress decreases in plasma acyl ghrelin levels and CDDP-induced anorexia.[14] Our use of rikkunshito in tumor-bearing rats was effective not only against anorexia–cachexia, but also for promoting survival, particularly in combination with chemotherapy. Daily administration of a 5-HT2c receptor antagonist failed to prolong survival, suggesting that a sensitizing effect on the GHS-R may be essential for ameliorating ghrelin resistance in anorexia–cachexia in the long term. Pancreatic cancer patients generally respond poorly to chemotherapy,

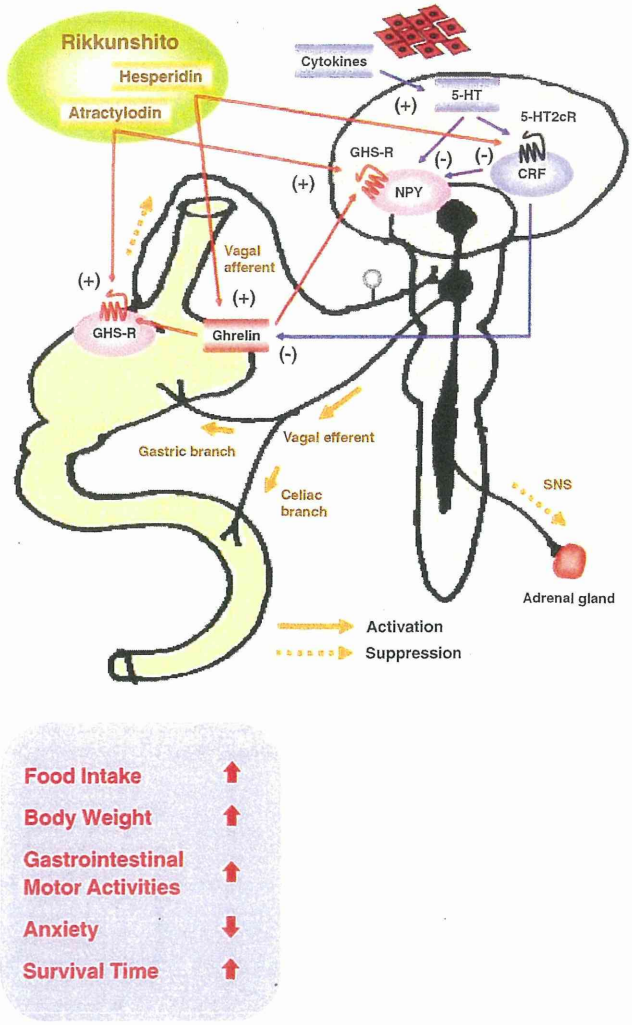

Figure 5 Ghrelin signaling and cancer anorexia–cachexia. Hypothalamic corticotropin-releasing factor (CRF) neurons are activated by cytokines through serotonin (5-HT) and the 5-HT 2c receptor (5-HT2cR), which shows functional divergence.[15] Our data demonstrate the existence of a novel 5-HT-CRF neuronal pathway that inhibits ghrelin secretion and has a pathogenetic role in cancer anorexia–cachexia syndrome. The traditional herbal medicine rikkunshito improves anorexia, weight loss, gastrointestinal (GI) dysmotility, anxiety-related behavior and survival. Rikkunshito and its active component hesperidin stimulate ghrelin secretion from stomach by interrupting this 5-HT-CRF pathway via 5-HT2cR antagonism. Another active component atractylodin potentiates the action of ghrelin by presumably allosterically sensitizing the GHS-R on the vagal afferent terminals of stomach or neuropeptide Y (NPY) neurons of the hypothalamic arcuate nucleus (ARC). The 5-HT2cR antagonist improved anorexia–cachexia in the short term, but failed to improve survival. Thus, both the release of ghrelin and the potentiation of ghrelin/GHS-R signaling are important for mitigating ghrelin insufficiency and resistance, which are characteristics of cancer anorexia–cachexia syndrome.

resulting in a higher frequency of anorexia–cachexia. We found that the median survival of pancreatic cancer patients treated with gemcitabine was prolonged by the addition of rikkunshito, particularly for those with ascites. These results suggest that rikkunshito may be useful in clinical practice for cachectic cancer patients via its dual action on ghrelin secretion and receptor sensitization.

In conclusion, our study demonstrates that cancer anorexia–cachexia is mediated by decreased ghrelin/GHS-R signaling as a result of excessive hypothalamic interactions of 5-HT and CRF through the 5-HT2cR. Decreased ghrelin signaling is an important integrated mechanism linking anorexia, body weight loss, GI dysmotility, anxiety-related behavior and decreased survival. Potentiation of ghrelin signaling introduces a novel approach for the treatment of cancer anorexia–cachexia, which is characterized by ghrelin insufficiency and resistance.

Conflict of interest

AI has received grant support from Tsumura & Co. The remaining authors have nothing to declare.

Acknowledgements. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health Labour and Welfare of Japan.

Author contributions. AA and AI supervised the project and designed experiments. NF, AN, TS, TH and YK performed the experiments. YU, KM, To Y, YM and US performed the cellular analysis. TaY analyzed the survival data in cancer patients. NF, AA and AI wrote the paper. All authors critically discussed the results and the manuscript as a whole.

Ethics statement. All experimental procedures were performed according to the 'Guidelines for the Care and Use of Laboratory Animals' approved by the Institutional Review Boards of the National Cancer Center Research Institute, the Jichi Medical University and Tsumura. Humane end points were chosen to minimize or terminate pain or distress in the animals via euthanasia, rather than waiting for death as an end point. All research involving human participants was approved by the Institutional Review Board of Chiba Cancer Center. The study did not require patient consent, as it was a retrospective analysis. However, participants gave written or verbal consent. Data were anonymized and no patient identifying information was included. All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki.

1. Tisdale MJ. Biology of cachexia. *J Natl Cancer Inst* 1997; **89**: 1763–1773.
2. Mantovani G, Maccio A, Massa E, Madeddu C. Managing cancer-related anorexia/cachexia. *Drugs* 2001; **61**: 499–514.
3. Inui A. Eating behavior in anorexia nervosa—an excess of both orexigenic and anorexigenic signalling? *Mol Psychiatry* 2001; **6**: 620–624.
4. Perboni S, Inui A. Anorexia in cancer: role of feeding-regulatory peptides. *Philos Trans R Soc Lond Ser B* 2006; **361**: 1281–1289.
5. Morley JE, Farr SA. Cachexia and neuropeptide Y. *Nutrition* 2008; **24**: 815–819.
6. Inui A. Cancer anorexia–cachexia syndrome: current issues in research and management. *CA Cancer J Clin* 2002; **52**: 72–91.
7. Jatoi A, Yamashita J, Slotan JA, Novotny PJ, Windschitl HE, Loprinzi CL. Does megastrol acetate down-regulate interleukin-6 in patients with cancer-associated anorexia and weight loss? A North Central Cancer Treatment Group Investigation. *Support Care Cancer* 2002; **10**: 71–75.
8. Nelson KA. The cancer anorexia–cachexia syndrome. *Semin Oncol* 2000; **27**: 64–68.
9. North CS, Hong BA, Alpers DH. Relationship of functional gastrointestinal disorders and psychiatric disorders: implications for treatment. *World J Gastroenterol* 2007; **13**: 2020–2027.
10. Levy RL, Olden KW, Naliboff BD, Bradley LA, Francisconi C, Drossman DA *et al.* Psychosocial aspects of the functional gastrointestinal disorders. *Gastroenterology* 2006; **130**: 1447–1458.
11. Smagin GN, Dunn AJ. The role of CRF receptor subtypes in stress-induced behavioural responses. *Eur J Pharmacol* 2000; **405**: 199–206.
12. Inui A. Feeding and body-weight regulation by hypothalamic neuropeptides-mediation of the actions of leptin. *Trends Neurosci* 1999; **22**: 62–67.
13. Chen CY, Fujimiya M, Laviano A, Chang FY, Lin HC, Lee SD. Modulation of ingestive behavior and gastrointestinal motility by ghrelin in diabetic animals and humans. *J Chin Med Assoc* 2010; **73**: 225–229.
14. Takeda H, Sadakane C, Hattori T, Katsurada T, Ohkawara T, Nagai K *et al.* Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. *Gastroenterology* 2008; **134**: 2004–2013.
15. Fujitsuka N, Asakawa A, Hayashi M, Sameshima M, Amitani H, Kojima S *et al.* Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT2c receptor and acyl ghrelin. *Biol Psychiatry* 2009; **65**: 748–759.
16. Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W. Corticotropin releasing factor produces behavioural activation in rats. *Nature* 1982; **297**: 331–333.
17. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N *et al.* Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337–345.
18. Kohno D, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with NPY-containing neurons in the rat arcuate nucleus: Ca^{2+} signaling via protein kinase A- and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 2003; **52**: 948–956.
19. Kohno D, Nakata M, Maekawa F, Fujiwara K, Maejima Y, Kuramochi M *et al.* Leptin suppresses ghrelin-induced activation of neuropeptide Y neurons in the arcuate nucleus via phosphatidylinositol 3-kinase- and phosphodiesterase 3-mediated pathway. *Endocrinology* 2007; **148**: 2251–2263.
20. Shimizu Y, Nagaya N, Isobe T, Imazu M, Okumura H, Hosoda H *et al.* Increased plasma ghrelin level in lung cancer cachexia. *Clin Cancer Res* 2003; **9**: 774–778.
21. Garcia JM, Garcia-Touza M, Hijazi RA, Taffet G, Epner D, Mann D *et al.* Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. *J Clin Endocrinol Metab* 2005; **90**: 2920–2926.
22. Hanada T, Toshinai K, Date Y, Kajimura N, Tsukada T, Hayashi Y *et al.* Upregulation of ghrelin expression in cachectic nude mice bearing human melanoma cells. *Metabolism* 2004; **53**: 84–88.
23. Flier JS. Clinical review 94: What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab* 1998; **83**: 1407–1413.
24. Schwartz MW, Dallman MF, Woods SC. Hypothalamic response to starvation: implications for the study of wasting disorders. *Am J Physiol* 1995; **269**: 949–957.
25. Schwartz MW, Seeley RJ. Seminars in medicine of the Beth Israel Deaconess Medical Center. Neuroendocrine responses to starvation and weight loss. *N Engl J Med* 1997; **336**: 1802–1811.
26. Sakurada S, Shido O, Sugimoto N, Hiratsuka Y, Yoda T, Kanosue K. Autonomic and behavioural thermoregulation in starved rats. *J Physiol* 2000; **526**: 417–424.
27. Zhao Z, Sakata I, Okubo Y, Koike K, Kangawa K, Sakai T. Gastric leptin, but not estrogen and somatostatin, contributes to the elevation of ghrelin mRNA expression level in fasted rats. *J Endocrinol* 2008; **196**: 529–538.
28. Turrin NP, Ilyin SE, Gayle DA, Plata-Salaman CR, Ramos EJ, Laviano A *et al.* Interleukin-1beta system in anorectic catabolic tumor-bearing rats. *Curr Opin Clin Metab Care* 2004; **7**: 419–426.
29. Gyengesi E, Gyengesi E, Liu ZW, D'Agostino G, Gan G, Horvath TL *et al.* Corticosterone regulates synaptic input organization of POMC and NPY/AgRP neurons in adult mice. *Endocrinology* 2010; **151**: 5395–5402.
30. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C *et al.* Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* 2004; **89**: 2832–2836.
31. Laferrere B, Abraham C, Russell CD, Bowers CY. Growth hormone releasing peptide-2 (GHRP-2), like ghrelin, increases food intake in healthy men. *J Clin Endocrinol Metab* 2005; **90**: 611–614.
32. Kusunoki H, Haruma K, Hata J, Ishii M, Kamada T, Yamashita N *et al.* Efficacy of rikkunshito, a traditional Japanese medicine (Kampo), in treating functional dyspepsia. *Intern Med* 2010; **49**: 2195–2202.
33. Takahashi T, Endo S, Nakajima K, Souma Y, Nishida T. Effect of rikkunshito, a Chinese herbal medicine, on stasis in patients after pylorus-preserving gastrectomy. *World J Surg* 2009; **33**: 296–302.
34. Oyachi N, Takano K, Hasuda N, Arai H, Koshizuka K, Matsumoto M. Effects of Rikkunshito on infantile hypertrophic pyloric stenosis, refractory to atropine. *Pediatr Int* 2008; **50**: 581–583.
35. Oka T, Tamagawa Y, Hayashida S, Kaneda Y, Kodama N, Tsuji S. Rikkunshito attenuates adverse gastrointestinal symptoms induced by fluvoxamine. *Biopsychosoc Med* 2007; **1**: 21.
36. Yagi M, Homma S, Kubota M, Iinuma Y, Kanada S, Kinoshita Y *et al.* The herbal medicine Rikkunshito stimulates and coordinates the gastric myoelectric activity in post-operative dyspeptic children after gastrointestinal surgery. *Pediatr Surg Int* 2004; **19**: 760–765.
37. Kawahara H, Okuyama H, Nose K, Nakai H, Yoneda A, Kubota A *et al.* Physiological and clinical characteristics of gastroesophageal reflux after congenital diaphragmatic hernia repair. *J Pediatr Surg* 2010; **45**: 2346–2350.

38. Matsumura T, Arai M, Yonemitsu Y, Maruoka D, Tanaka T, Suzuki T *et al.* The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. *J Gastroenterol* 2010; **45**: 300–307.
39. Yakabi K, Kurosawa S, Tamai M, Yuzurihara M, Nahata M, Ohno S *et al.* Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction. *Regul Pept* 2010; **161**: 97–105.
40. Halford JC, Harrold JA, Boyland EJ, Lawton CL, Blundell JE. Serotonergic drugs: effects on appetite expression and use for the treatment of obesity. *Drugs* 2007; **67**: 27–55.
41. Vickers SP, Dourish CT, Kennett GA. Evidence that hypophagia induced by *d*-fenfluramine and *d*-norfenfluramine in the rat is mediated by 5-HT2C receptors. *Neuropharmacology* 2001; **41**: 200–209.
42. Vickers SP, Easton N, Webster LJ, Wyatt A, Bickerdike MJ, Dourish CT *et al.* Oral administration of the 5-HT2C receptor agonist, mCPP, reduces body weight gain in rats over 28 days as a result of maintained hypophagia. *Psychopharmacology* 2003; **167**: 274–280.
43. Wang W, Danielsson A, Svanberg E, Lundholm K. Lack of effects by tricyclic antidepressant and serotonin inhibitors on anorexia in MCG 101 tumor bearing mice with eicosanoid-related cachexia. *Nutrition* 2003; **19**: 47–53.
44. Makarenko IG, Meguid MM, Gatto L, Goncalves CG, Ramos EJ, Chen C *et al.* Hypothalamic 5-HT1B-receptor changes in anorectic bearing rats. *Neurosci Lett* 2005; **376**: 71–75.
45. Makarenko IG, Meguid MM, Gatto L, Chen C, Ugrumov MV. Decreased NPY innervation of the hypothalamic nuclei in rats with cancer anorexia. *Brain Res* 2003; **961**: 100–108.
46. Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L. Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J Comp Neurol* 1995; **351**: 357–373.
47. Heisler LK, Pronchuk N, Nonogaki K, Zhou L, Raber J, Tung L *et al.* Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. *J Neurosci* 2007; **27**: 6956–6964.
48. Shintani F, Kanba S, Nakaki T, Nibuya M, Kinishita N, Suzuki E *et al.* Interleukin-1beta augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. *J Neurosci* 1993; **13**: 3574–3581.
49. Chuang JC, Zigman JM. Ghrelin's roles in stress, mood, and anxiety regulation. *Int J Pept* 2010; **id**: 460549.
50. Kamiji MM, Inui A. Neuropeptide Y receptor selective ligands in the treatment of obesity. *Endocr Rev* 2007; **28**: 664–684.
51. Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H *et al.* The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120–1128.
52. Fujino K, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol* 2003; **550**: 227–240.
53. Muroya S, Yada T, Shioda S, Takigawa M. Glucose-sensitive neurons in the rat arcuate nucleus contain neuropeptide Y. *Neurosci Lett* 1999; **264**: 113–116.

Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)

Critical Reviews™ in Oncogenesis, 17(3), 277–284 (2012)

Efficacy of Ghrelin in Cancer Cachexia: Clinical Trials and a Novel Treatment by Rikkunshito

Naoki Fujitsuka[1,2], Akihiro Asakawa[1], Haruka Amitani[1], Tomohisa Hattori[2], & Akio Inui[1,*]

[1]Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8520, Japan, [2]Tsumura Research Laboratories, Ibaraki 300-1192, Japan

*Address all correspondence to: Akio Inui, Professor, Kagoshima University Graduate School of Medical and Dental Sciences, Department of Psychosomatic Internal Medicine, 8-35-1 Sakuragaoka, Kagoshima, 890-8520, Japan; Tel. & Fax: +81-99-275-5748; inui@m.kufm.kagoshima-u.ac.jp

ABSTRACT: Cachexia is characterized by decreased food intake, increased energy expenditure, and muscle wasting. It is observed in 80% of patients with advanced-stage cancer and is a major source of decreased quality of life and increased morbidity and mortality in cancer patients. Ghrelin plays an important role in stimulating hunger and maintaining energy homeostasis and is the first-line treatment option for cancer cachexia. Several studies in rodent models and clinical trials have demonstrated that ghrelin or ghrelin receptor (GHS-R) agonists are effective in the treatment of cancer cachexia; however, further large-scale long-term clinical trials are needed to confirm sustained effects. Recently, the traditional Japanese medicine rikkunshito has been shown to increase food intake in rats with cancer or administered chemotherapeutics. The orexigenic effect of rikkunshito is involved in the stimulation of endogenous ghrelin secretion by blocking the serotonin (5-HT) 2b/2c receptor pathway and the enhancement of GHS-R activity. A potentiator of ghrelin signaling such as rikkunshito may represent a novel approach for the treatment of cancer cachexia.

KEY WORDS: Ghrelin, cancer, cachexia, GHS-R, Rikkunshito

ABBREVIATIONS

AgRP: agouti-related peptide; BMI: body mass index; CCK: cholecystokinin; GH: growth hormone; GHS-R: growth hormone secretagogue receptor; GI: gastrointestinal; GOAT: ghrelin O-acyltransferase; IGF-1: insulin-like growth factor-1; IL-1β: interleukin-1β; NPY: neuropeptide Y; QOL: quality of life; TNF-α: tumor necrosis factor-α.

I. INTRODUCTION

Cachexia is characterized by decreased food intake, weight loss, and muscle tissue wasting and is observed in 80% of patients with advanced-stage cancer.[1, 2] Cancer cachexia not only is a major source of decreased quality of life (QOL) but also increases morbidity and mortality in cancer patients. A persistent loss of appetite leads to a progressive depletion of body energy stores; accordingly, the development of anorexia is frequently associated with cachexia. Cancer cachexia is predominantly dependent on an imbalance between anorexigenic and orexigenic signals induced by proinflammatory cytokines that are either produced by cancer cells or released by the host immune system in response to the cancer.[3] Weight loss is a potent stimulus of food intake in healthy humans and animals, but not in individuals with cancer. Consequently, the improvement of neurochemical mechanisms regulating appetite and energy homeostasis is critically important for the treatment of cancer cachexia.

The ghrelin system is involved in eliciting feeding, inducing adiposity, and regulating energy expenditure and body weight.[4,5] Ghrelin plays an important role in triggering the adaptive response to starvation. In addition, ghrelin has much broader physiologic functions, including roles in growth hormone secretion,[6] gastrointestinal (GI) motility,[7] and suppressing inflammation.[8] Thus, ghrelin is expected be an

0893-9675/12/$35.00

effective therapy for lean patients with cachexia. Recent reports[9–11] have indicated that the traditional Japanese medicine rikkunshito, which stimulates the secretion of endogenous ghrelin by blocking 5-HT2b/2c receptors and enhances GHS-R activity in rats, increases food intake in rats with cancer or undergoing chemotherapy. The purpose of this article is to review the current medical treatment of cancer cachexia, in particular focusing on ghrelin and ongoing research.

II. GHRELIN PATHOPHYSIOLOGY

Ghrelin is a 28-amino-acid peptide, first isolated from the stomachs of humans and rats, that acts as a natural ligand for the growth hormone secretagogue receptor (GHS-R).[6] Ghrelin is mainly produced by the P/D1 cells lining the fundus of the stomach in humans and the X/A-like cells in rodents. Ghrelin mRNA is predominantly expressed in the stomach, but small amounts are seen in several tissues.[12] Acylation of Ser-3 by the addition of *n*-octanoic acid is essential for the biological activity of ghrelin via the GHS-R. Acyl modification of ghrelin is performed by the polytopic membrane-bound enzyme ghrelin O-acyltransferase (GOAT).[13] Once released, acyl ghrelin has a short half-life of approximately 10 min in the general circulation before being converted to desacyl ghrelin.[14]

Ghrelin enhances growth hormone secretion, but it has much broader physiologic functions, including appetite, GI motility, inflammation, circulation, and cell proliferation.[4–8,15] Plasma ghrelin levels increase in response to prolonged fasting and decrease rapidly after feeding. Weight loss is a potent stimulus of food intake in healthy humans and animals, and ghrelin secretion increases under conditions of negative energy balance such as starvation. Plasma ghrelin levels are higher in subjects with a low body mass index (BMI) compared with normal- or high-BMI subjects.[16] Accordingly, ghrelin is thought to be an orexigenic peptide that maintains energy homeostasis and provides a defense against starvation.

Administration of ghrelin has been shown to increase the gene expression of the orexigenic neuropeptides, namely, neuropeptide Y (NPY) and agouti-related peptide (AgRP) and to decrease the expression of the anorexigenic neuropeptide pro-opiomelanocortin.[17] Central or peripheral administration of ghrelin strongly stimulates food intake in animals.[4,5] Continuous intracerebroventricular administration of ghrelin induces food intake and increases fat mass, leading to weight gain.[18] Intravenous administration of ghrelin to healthy humans increased energy intake from a buffet lunch by 28%, and visual analog scores for appetite also increased under these conditions.[19] Administration of a single dose of GHS-R agonists to healthy volunteers has resulted in an increase in food intake.[14] These results suggest the possible clinical applications of ghrelin as a potent stimulator of appetite.

III. EFFICACY OF GHRELIN ON CANCER CACHEXIA

Increased circulating ghrelin levels have been observed in underweight patients and rodents with malignancy-associated cachexia.[20] Garcia et al. reported that ghrelin levels were significantly elevated in cachectic subjects compared with noncachectic cancer controls and noncancer controls (141, 91, and 78 pg/mL, respectively).[21] These elevations may be a compensatory response reflecting the state of negative energy balance. However, this phenomenon has been called "ghrelin resistance" due to a failure of the adaptive feeding response by ghrelin, which is robust in normal animals and subjects.[22–24] Cancer cachexia is associated with high concentrations of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).[25] Proinflammatory cytokines induce the release of 5-HT, leptin, cholecystokinin (CCK), peptides derived from the glucagon precursor, and insulin, all of which are hormones that act as satiety signals.[26–28] In particular, the 5-HT concentration in the hypothalamus is increased in humans and animals with cancer.[29,30] Makarenko et al. demonstrated that NPY and dopamine concentrations decrease while serotonin concentration increases in the paraventricular nucleus at the onset of anorexia in tumor-bearing rats.[31]

Nevertheless, ghrelin administration in rodent models of cancer cachexia led to a significant increase in food intake. Hanada et al.[32] and Wang et al.[33] demonstrated that twice-daily intraperitoneal administration of ghrelin (800 nmol/kg/day) to melanoma-bearing nude mice improved food intake and weight gain 5 or 6 days after treatment. DeBoer et al. demonstrated that the administration of ghrelin and a GHS-R agonist (BIM-28131) to tumor-bearing rats with cachexia as a continuous infusion (500 nmol/kg/day) via an osmotic minipump for 5 days of treatment resulted in increased food intake and weight gain and inhibited the loss of lean body mass.[34] These findings suggest that high plasma concentrations of ghrelin may overcome resistance to the appetite-stimulating effects of the endogenous peptide in the short term. In addition, ghrelin inhibits the production of anorectic proinflammatory cytokines, including IL-1β, IL-6, and TNF-α.[35] DeBoer et al. demonstrated that ghrelin-treated animals exhibited a significant decrease in the expression of IL-1 receptor-I transcript in the hypothalamus and brainstem. The combination of these actions suggests that ghrelin has benefits for the treatment of cachexia.[34]

IV. CLINICAL TRIALS

Several randomized, double-blinded, placebo-controlled trials have demonstrated that ghrelin or an GHS-R agonist effectively increases food intake and lean body mass in cachectic patients with cancer. Neary et al. demonstrated that energy intake from a buffet lunch was increased by 31% during ghrelin infusion (5 pmol/kg/min for 180 min) compared with the saline control in seven cancer patients.[36] Analysis of the visual analog score revealed a significant increase of 23% in meal appreciation on the ghrelin administration day. No adverse effects were observed. There was no evidence of a compensatory decrease in food intake after ghrelin treatment as assessed by a 24-h food diary. Strasser et al.[37] studied 21 cancer patients who were randomized to receive ghrelin on days 1 and 8 and placebo on days 4 and 11 or vice versa, given intravenously over a 60-min period before lunch. Ten patients received 2 mg/kg of ghrelin (lower dose) ; 11 received 8 mg/kg of ghrelin (higher dose). At day 8, 81% of patients preferred ghrelin to placebo against 63% at the end of study. Nutritional intake and eating-related symptoms were not significantly different between ghrelin and placebo. Ghrelin was well tolerated and safe in patients with advanced cancer. For safety, tolerance, and patients' preference for treatment, no difference was observed between the lower- and higher-dose groups. Garcia et al.[38] demonstrated that the GHS-R agonist RC-1291 orally administered over a 12-week period to 81 patients with a variety of cancers resulted in an increase in total body mass, lean body mass, and handgrip strength. No significant differences were found in QOL.

An important concern regarding the use of ghrelin in cancer cachexia is that ghrelin may stimulate tumor growth via GHS-Rs expressed in cancer cells or via growth factors such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1).[39] However, there are conflicting data about the possible role of ghrelin in oncogenesis,[40] and no *in vivo* studies have examined the differences in tumor growth after ghrelin or GHS treatment. Further large-scale, long-term clinical trials are required to determine the efficacy and safety of ghrelin or GHS-R agonists on cancer cachexia.

V. A NOVEL APPROACH FOR THE TREATMENT OF CANCER CACHEXIA

The traditional Japanese medicine *rikkunshito* is widely prescribed in patients exhibiting upper GI symptoms such as functional dyspepsia and gastroesophageal reflux.[41–45] Several reports have demonstrated that the oral administration of rikkunshito increases plasma ghrelin levels in humans and rodents and effectively improves food intake and modulates GI motility.[9,10,46] Takeda et al. demonstrated that rikkunshito ameliorated cisplatin-induced anorexia in rats by inhibiting the decreased concentration of circulating ghrelin.[9] Cisplatin is widely used in clinical practice; however, adverse reactions to the production of excess 5-HT lead to the discontinuation of chemotherapy in cancer patients. The 5-HT produced during treatment

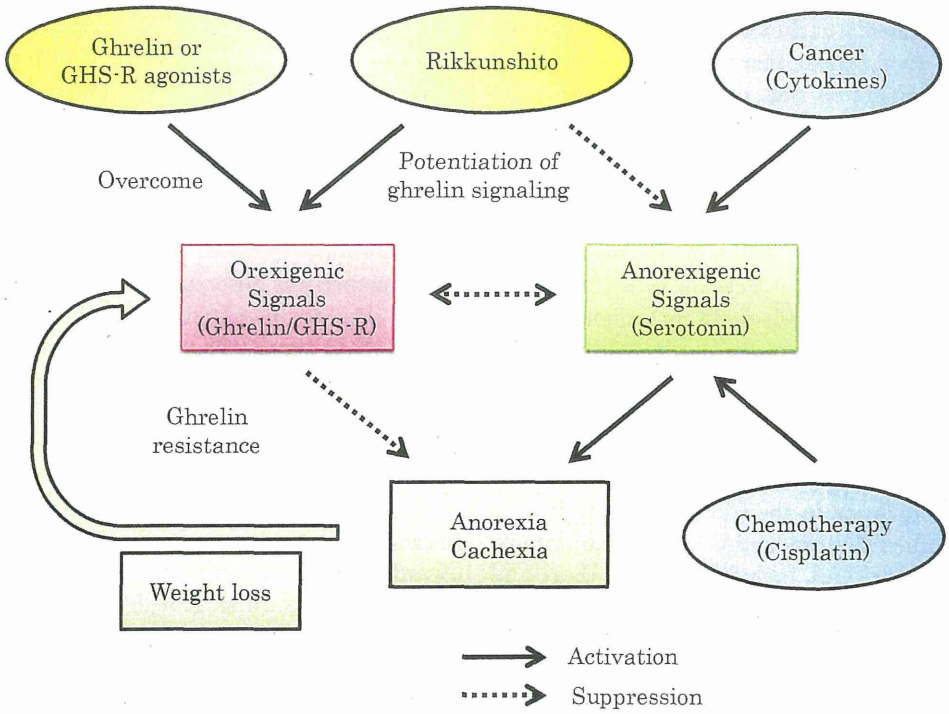

FIGURE 1. Pathophysiological role of ghrelin signaling in cancer cachexia. Cancer anorexia-cachexia is predominantly dependent on an imbalance between anorexigenic and orexigenic signals. In the hypothalamus, increased levels of proinflammatory cytokines produced by cancer cells play a role in activating the anorexigenic signals and inhibiting the orexigenic signals. Serotonin (5-HT) produced during treatment with cisplatin stimulates the 5-HT2b/2c receptors, resulting in decreased hypothalamic and peripheral ghrelin secretion. In patients and rodents with cancer cachexia, circulating ghrelin levels increase due to the adaptive response to weight loss; however, ghrelin resistance is induced by excessive hypothalamic anorexigenic activity. Treatment with ghrelin or GHS-R agonists can overcome resistance to the appetite-stimulating effect of the endogenous ghrelin. Rikkunshito increases food intake in rats with cancer or chemotherapy by the stimulation of endogenous ghrelin secretion by blocking the 5-HT 2b/2c receptor pathway and the enhancement of GHS-R activity. A potentiator of ghrelin signaling such as rikkunshito may represent an additional novel approach for the treatment of cancer cachexia.

with cisplatin stimulates the 5-HT2b receptor in gastric smooth muscle and the 5-HT2c receptor in the central nervous system, resulting in decreased plasma ghrelin. Heptamethoxyflavone, hesperidin, and isoliquiritigenin (components of rikkunshito) have been shown to antagonize 5-HT2b/2c receptors and stimulate ghrelin secretion in cisplatin-treated rats, suggesting that these molecules play an important role in the improvement of appetite by rikkunshito. Our previous study[10] reported that the oral administration of rikkunshito to fenfluramine-treated rats increased plasma ghrelin levels, food intake, and gastric emptying time and restored GI dysmotility. Fenfluramine altered the fasted motor activities to become fed-like motor activities in the antrum and duodenum via the activation of the central 5-HT2c receptor, mediated by the ghrelin-NPY signaling pathway. These effects of rikkunshito in fenfluramine-treated rats were blocked by the GHS-R antagonist (D-Lys3)-GHRP-6, suggesting

that the increase in circulating ghrelin induced by rikkunshito leads to the improvement of anorexia and gastric function. Yakabi et al. also demonstrated that urocortin-induced reduction of food intake was restored by rikkunshito.[47]

A recent report has demonstrated that rikkunshito enhances hypothalamic ghrelin secretion.[48] In cisplatin-treated rats, hypothalamic ghrelin secretion was markedly reduced 24 and 48 h after cisplatin treatment, although plasma ghrelin levels were higher than in saline-treated rats due to the adaptive response to weight loss. Cisplatin-induced anorexia in the late phase is mediated through reduced hypothalamic ghrelin secretion. Cerebral 5-HT2c receptor activation partially induces decreases in hypothalamic ghrelin secretion, and rikkunshito suppresses cisplatin-induced anorexia by enhancing this secretion. In addition, rikkunshito and 5-HT2c receptor antagonists suppress cisplatin-induced anorexia by inhibiting the reduction of GHS-R1a gene expression in the hypothalamus.[49] The efficacy of rikkunshito in cisplatin-induced anorexia may reduce the risk of discontinuation of chemotherapy in cancer patients. In addition, Takeda et al. reported that rikkunshito improved aging-associated anorexia by inhibiting the reduced reactivity of the hypothalamic ghrelin receptor, which is caused by an increase in plasma leptin level.[50] The components of rikkunshito that inhibit phosphodiesterase type 3 mediation downstream of the leptin receptor have been identified.

Cancer cachexia is caused by multiple underlying mechanisms. Anorexigenic neurochemical mediators such as 5-HT, which increase in the hypothalamus in humans and animals with cancer, are predominantly involved.[25] The excessive hypothalamic anorexigenic activity may induce ghrelin resistance, which attenuates the adaptive feeding response by endogenous ghrelin in cancer cachexia. More recently, we found that rikkunshito improved anorexia, GI dysmotility, muscle wasting, and anxiety-related behavior and prolonged survival in tumor-bearing rats. This effect is mediated by the stimulation of ghrelin secretion and the enhancement of GHS-R activity.[11] These findings suggest that rikkunshito may be effective for ghrelin resistance such as cancer cachexia (Figure 1).

VI. CONCLUSION

Several rodent models and short-term clinical studies have demonstrated that ghrelin or GHS-R agonists are effective in the treatment of cancer cachexia. However, further large-scale long-term clinical trials are required to determine the efficacy and safety of ghrelin or GHS-R agonists in cancer cachexia. A potentiator of ghrelin signaling such as rikkunshito may represent an additional novel approach for the treatment of cancer cachexia.

REFERENCES

1. Tisdale MJ. Biology of cachexia. J Natl Cancer Inst. 1997 Dec 3;89(23):1763–73.
2. Mantovani G, Maccio A, Massa E, Madeddu C. Managing cancer-related anorexia/cachexia. Drugs. 2001;61(4):499–514.
3. Inui A. Cancer anorexia-cachexia syndrome: current issues in research and management. CA Cancer J Clin. 2002 Mar-Apr 52;(2):72–91.
4. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. Nature. 2001 Jan 11;409(6817):194–8.
5. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology. 2001 Feb 120;(2):337–45.
6. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999 Dec 9;402(6762):656–60.
7. Fujino K, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. J Physiol. 2003 Jul 1;550(Pt 1):227–40.
8. Granado M, Priego T, Martin AI, Villanua MA, Lopez-Calderon A. Anti-inflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats. Am J Physiol Endocrinol Metab. 2005 Mar 288;(3):E486–E492.
9. Takeda H, Sadakane C, Hattori T, Katsurada T, Ohkawara T, Nagai K, Asaka M. Rikkunshito,

an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. Gastroenterology. 2008 Jun 134;(7):2004–13.
10. Fujitsuka N, Asakawa A, Hayashi M, Sameshima M, Amitani H, Kojima S, Fujimiya M, Inui A. Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT2c receptor and acyl ghrelin. Biol Psychiatry. 2009 May 1;65(9):748–59.
11. Fujitsuka N, Asakawa A, Uezono Y, Minami K, Yamaguchi T, Niijima A, Yada T, Maejima Y, Sedbazar U, Sakai T, Hattori T, Kase Y, Inui A. Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival. Transl Psychiatry. 2011;1:e23.
12. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, Korbonits M. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab. 2002 Jun 87;(6):2988.
13. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell. 2008 Feb 8;132(3):387–96.
14. Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, Tada H, Miura K, Shimizu A, Fukushima M, Yokode M, Tanaka K, Kangawa K. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol. 2004 Apr 150;(4):447–55.
15. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev. 2005 Apr 85;(2):495–522.
16. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab. 2002 Jan 87;(1):240–44.
17. Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van der Ploeg LH, Howard AD, MacNeil DJ, Qian S. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology. 2004 Jun 145;(6):2607–12.
18. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000 Oct 19 407;(6806):908–13.
19. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. 2001 Dec 86;(12):5992.
20. Shimizu Y, Nagaya N, Isobe T, Imazu M, Okumura H, Hosoda H, Kojima M, Kangawa K, Kohno N. Increased plasma ghrelin level in lung cancer cachexia. Clin Cancer Res. 2003 Feb 9;(2):774–8.
21. Garcia JM, Garcia-Touza M, Hijazi RA, Taffet G, Epner D, Mann D, Smith RG, Cunningham GR, Marcelli M. Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. J Clin Endocrinol Metab. 2005 May 90;(5):2920–6.
22. Flier JS. Clinical review 94: What's in a name? In search of leptin's physiologic role. J Clin Endocrinol Metab. 1998 May 83;(5):1407–13.
23. Schwartz MW, Dallman MF, Woods SC. Hypothalamic response to starvation: implications for the study of wasting disorders. Am J Physiol. 1995 Nov 269;(5 Pt 2):R949–R957.
24. Schwartz MW, Seeley RJ. Seminars in medicine of the Beth Israel Deaconess Medical Center. Neuroendocrine responses to starvation and weight loss. N Engl J Med. 1997 Jun 19 336;(25):1802–11.
25. Perboni S, Inui A. Anorexia in cancer: role of feeding-regulatory peptides. Philos Trans R Soc Lond B Biol Sci. 2006 Jul 29;361(1471):1281–9.
26. Plata-Salaman CR. Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. Nutrition. 2000 Oct 16;(10):1009–12.
27. Shintani F, Kanba S, Nakaki T, Nibuya M, Kinoshita N, Suzuki E, Yagi G, Kato R, Asai M. Interleukin-1 beta augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. J Neurosci. 1993 Aug 13;(8):3574–81.
28. Laviano A, Gleason JR, Meguid MM, Yang ZJ, Cangiano C, Rossi Fanelli F. Effects of intra-VMN mianserin and IL-1ra on meal number in anorectic tumor-bearing rats. J Investig Med. 2000 Jan 48;(1):40–8.
29. Wang W, Danielsson A, Svanberg E, Lundholm K. Lack of effects by tricyclic antidepressant and serotonin inhibitors on anorexia in MCG 101 tumor-bearing mice with eicosanoid-related cachexia. Nutrition. 2003 Jan 19;(1):47–53.
30. Makarenko IG, Meguid MM, Gatto L, Goncalves CG, Ramos EJ, Chen C, Chen C, Ugrumov MV. Hypothalamic 5-HT1B-receptor changes in anorectic tumor bearing rats. Neurosci Lett. 2005 Mar 11 376;(2):71–5.

31. Makarenko IG, Meguid MM, Gatto L, Chen C, Ugrumov MV. Decreased NPY innervation of the hypothalamic nuclei in rats with cancer anorexia. Brain Res. 2003 Jan 24 961;(1):100–8.
32. Hanada T, Toshinai K, Kajimura N, Nara-Ashizawa N, Tsukada T, Hayashi Y, Osuye K, Kangawa K, Matsukura S, Nakazato M. Anti-cachectic effect of ghrelin in nude mice bearing human melanoma cells. Biochem Biophys Res Commun. 2003 Feb 7 301;(2):275–9.
33. Wang W, Andersson M, Iresjo BM, Lonnroth C, Lundholm K. Effects of ghrelin on anorexia in tumor-bearing mice with eicosanoid-related cachexia. Int J Oncol. 2006 Jun 28;(6):1393–400.
34. DeBoer MD, Zhu XX, Levasseur P, Meguid MM, Suzuki S, Inui A, Taylor JE, Halem HA, Dong JZ, Datta R, Culler MD, Marks DL. Ghrelin treatment causes increased food intake and retention of lean body mass in a rat model of cancer cachexia. Endocrinology. 2007 Jun 148;(6):3004–12.
35. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. J Clin Invest. 2004 Jul 114;(1):57–66.
36. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC, Bloom SR. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab. 2004 Jun 89;(6):2832–6.
37. Strasser F, Lutz TA, Maeder MT, Thuerlimann B, Bueche D, Tschop M, Kaufmann K, Holst B, Brändle M, von Moos R, Demmer R, Cerny T. Safety, tolerability and pharmacokinetics of intravenous ghrelin for cancer-related anorexia/cachexia: a randomised, placebo-controlled, double-blind, double-crossover study. Br J Cancer. 2008 Jan 29;98(2):300–8.
38. Garcia JM, Boccia RV, Graham C, Kumor K, Polvino W. A Phase II, randomized, placebo-controlled, double blind study of the efficacy and safety of RC-1291 for the treatment of cancer-cachexia. J Clin Oncol. ASCO Annual Meeting Proceedings Part I. Vol. 25, No. 18S (June 20 Supplement), 2007: 9133.
39. Murata M, Okimura Y, Iida K, Matsumoto M, Sowa H, Kaji H, Kojima M, Kangawa K, Chihara K. Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. J Biol Chem. 2002 Feb 15;277(7):5667–74.
40. Ghe C, Cassoni P, Catapano F, Marrocco T, Deghenghi R, Ghigo E, Muccioli G, Papotti M. The antiproliferative effect of synthetic peptidyl GH secretagogues in human CALU-1 lung carcinoma cells. Endocrinology. 2002 Feb 143;(2):484–91.
41. Kusunoki H, Haruma K, Hata J, Ishii M, Kamada T, Yamashita N, Honda K, Inoue K, Imamura H, Manabe N, Shiotani A, Tsunoda T. Efficacy of Rikkunshito, a traditional Japanese medicine (Kampo), in treating functional dyspepsia. Intern Med. 2010 49;(20):2195–202.
42. Suzuki H, Inadomi JM, Hibi T. Japanese herbal medicine in functional gastrointestinal disorders. Neurogastroenterol Motil. 2009 Jul 21;(7):688–96.
43. Kawahara H, Okuyama H, Nose K, Nakai H, Yoneda A, Kubota A, Fukuzawa M. Physiological and clinical characteristics of gastroesophageal reflux after congenital diaphragmatic hernia repair. J Pediatr Surg. 2010 Dec 45;(12):2346–50.
44. Johnson DA, Levy BH, 3rd. Evolving drugs in gastroesophageal reflux disease: pharmacologic treatment beyond proton pump inhibitors. Expert Opin Pharmacother. 2010 Jun 11;(9):1541–8.
45. Miwa H, Koseki J, Oshima T, Kondo T, Tomita T, Watari J, Matsumoto T, Hattori T, Kubota K, Iizuka S. Rikkunshito, a traditional Japanese medicine, may relieve abdominal symptoms in rats with experimental esophagitis by improving the barrier function of epithelial cells in esophageal mucosa. J Gastroenterol. 2010 May 45;(5):478–87.
46. Matsumura T, Arai M, Yonemitsu Y, Maruoka D, Tanaka T, Suzuki T, Yoshikawa M, Imazeki F, Yokosuka O. The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. J Gastroenterol. 2010 Mar 45;(3):300–7.
47. Yakabi K, Noguchi M, Ohno S, Ro S, Onouchi T, Ochiai M, Takabayashi H, Takayama K, Harada Y, Sadakane C, Hattori T. Urocortin 1 reduces food intake and ghrelin secretion via CRF2 receptors. Am J Physiol Endocrinol Metab. 2011 Jul 301;(1): E72–E82.
48. Yakabi K, Sadakane C, Noguchi M, Ohno S, Ro S, Chinen K, Aoyama T, Sakurada T, Takabayashi H, Hattori T. Reduced ghrelin secretion in the hypothalamus of rats due to cisplatin-induced anorexia. Endocrinology. 2010 Aug 151;(8):3773–82.

49. Yakabi K, Kurosawa S, Tamai M, Yuzurihara M, Nahata M, Ohno S, Ro S, Kato S, Aoyama T, Sakurada T, Takabayashi H, Hattori T. Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction. Regul Pept. 2010 Apr 9;161(1–3):97–105.

50. Takeda H, Muto S, Hattori T, Sadakane C, Tsuchiya K, Katsurada T, Ohkawara T, Oridate N, Asaka M. Rikkunshito ameliorates the aging-associated decrease in ghrelin receptor reactivity via phosphodiesterase III inhibition. Endocrinology. 2010 Jan 151;(1):244–252.

CHAPTER EIGHTEEN

Ghrelin and Gastrointestinal Movement

Naoki Fujitsuka*,†, Akihiro Asakawa*, Haruka Amitani*, Mineko Fujimiya‡, Akio Inui*,1

*Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
†Tsumura Research Laboratories, Ibaraki, Japan
‡Department of Anatomy, Sapporo Medical University School of Medicine, Hokkaido, Japan
[1]Corresponding author: e-mail address: inui@m.kufm.kagoshima-u.ac.jp

Contents

1. Introduction 290
2. The Role of Ghrelin in Gastroduodenal Motility 291
   2.1 The strain-gauge force-transducer measurement of gastroduodenal motility in conscious rats and mice 291
   2.2 The manometric measurement of gastroduodenal motility in conscious rats and mice 293
   2.3 Measurement of gastroduodenal motility in conscious house musk shrews (*S. murinus*) 295
3. The GI Motor Effect of Ghrelin Mediated by the Gut–Brain Axis 296
   3.1 The brain mechanism responsible for mediating GI motility 296
   3.2 Ghrelin and GI disorders 298
4. Summary 299
Acknowledgments 299
References 299

Abstract

Ghrelin is a potent stimulant for gastric emptying and gastrointestinal (GI) movement. Clinically, it has been reported that the intravenous administration of ghrelin accelerates the rate of gastric emptying and induces gastric phase III contractions of the migrating motor complex in healthy volunteers. Recent technical advances in the measurement of GI motility in conscious small animals, including rats, mice, and the house musk shrew (*Suncus murinus*), have helped to elucidate the precise mechanism of action of ghrelin. Intravenous administration of ghrelin induces fasted motor activities with phase III-like contractions of the migrating motor complex in the antrum and duodenum in animals. These effects of ghrelin are mediated by activating the hypothalamic orexigenic neuropeptide Y neuron through ghrelin receptors located at the vagal afferent terminal. Stress hormone and anorexigenic peptides cause the disruption of fasted motor activity and induce fed-like motor activity. Ghrelin and

Methods in Enzymology, Volume 514
ISSN 0076-6879
http://dx.doi.org/10.1016/B978-0-12-381272-8.00018-0

the ghrelin signal potentiator rikkunshito successfully restore fed-like motor activities to fasted activities in fenfluramine-treated rats and in a cancer anorexia–cachexia animal model. These findings suggest that ghrelin can be expected to be a therapeutic target for GI disorders.

1. INTRODUCTION

Ghrelin is a potent stimulant of gastric emptying and gastrointestinal (GI) motility. In humans in the fasted state, cyclic changes in contraction waves known as the migrating motor complex (MMC) are observed in the GI tract (Vantrappen et al., 1977). The MMC consists of three phases: a period of motor quiescence (phase I), a period of irregular contractions (phase II), and a period of clustered potent contractions (phase III). These phases are observed at regular intervals of 90–120 min in humans. Clinically, ghrelin accelerates the rate of gastric emptying (Levin et al., 2006) and induces gastric phase III contractions in healthy volunteers (Bisschops, 2008; Tack et al., 2006). The same findings regarding GI motility in animal models have been reported following free-moving, conscious animal experiments. These experiments provide more physiological information than other approaches to estimating GI motility, which is regulated by the brain–gut interaction (Inui et al., 2004). Notably, the dog is a popular model for GI motility research. An early study (Itoh et al., 1976) showed that motilin induces gastric phase III contractions in dogs. However, there have been few reports showing that ghrelin, which has a structural resemblance to motilin, has an effect on the digestive tract in dogs (Ohno et al., 2010).

Recent technical advances have permitted the measurement of GI motility in conscious small animals, including rats, mice, and house musk shrews (*S. murinus*), using manometric methods (Ataka et al., 2008; Fujino et al., 2003; Tanaka et al., 2009) or force-transducer implantation (Ariga et al., 2007; Fujitsuka et al., 2009; Sakahara et al., 2010; Zheng et al., 2009a). These studies have demonstrated that ghrelin induces a fasted motor pattern and augments the motility of the antrum and duodenum in the fed or fasted state of healthy animals through brain–gut interactions. Moreover, ghrelin and rikkunshito have been shown to improve gastric emptying and GI motility in animal models of GI disorder. Rikkunshito, a traditional Japanese herbal (Kampo) medicine, potentiates ghrelin

signaling (Fujitsuka et al., 2011; Takeda et al., 2008, 2010; Yakabi et al., 2010) and is widely prescribed for patients exhibiting functional dyspepsia (Kusunoki et al., 2010). In this section, the role of ghrelin in GI motility and the methods for the measurement of GI motility in experimental animals are introduced.

2. THE ROLE OF GHRELIN IN GASTRODUODENAL MOTILITY

2.1. The strain-gauge force-transducer measurement of gastroduodenal motility in conscious rats and mice

The role of ghrelin in the control of gastroduodenal motility was evaluated in free-moving, conscious rats using a strain-gauge force-transducer method (Fig. 18.1A). In fasted rats, cyclic changes in contraction waves were detected in both the antrum and duodenum; these waves included a quiescent period (phase I-like contractions) followed by a group of contractions (phase III-like contractions) (Fig. 18.1B). Phase III-like contractions of the antrum occur periodically at intervals of approximately 10 min, and most of these contractions appear to occur in conjunction with phase III-like contractions of the duodenum. Circulating ghrelin levels in fasted rats fluctuate; the peaks of these fluctuations are highly associated with phase III-like contractions in the antrum (Ariga et al., 2007; Fujitsuka et al., 2009).

Intravenous administration of ghrelin to fasted rats immediately potentiates the fasted motor activity and increases the motility index (MI) and the frequency of phase III-like contractions in the antrum and duodenum (Fig. 18.1C). The physiological fasted motor activity decreases with the administration of the growth-hormone secretagogue receptor (ghrelin receptor) antagonist (D-Lys^3) GHRP-6. Exogenous ghrelin eliminates the fed motor pattern, which is irregular contractions of high frequency caused by feeding, and produces a fasted motor pattern (Fig. 18.1B and D) (Fujitsuka et al., 2009). Gastric motility in the physiological fed and fasted states of conscious mice has also been measured successfully by a method involving the implantation of a transducer in the mouse stomach (Zheng et al., 2009a). The protocol presented below has been used to measure gastroduodenal motility in conscious rats using the strain-gauge force-transducer method (Fig. 18.1A).

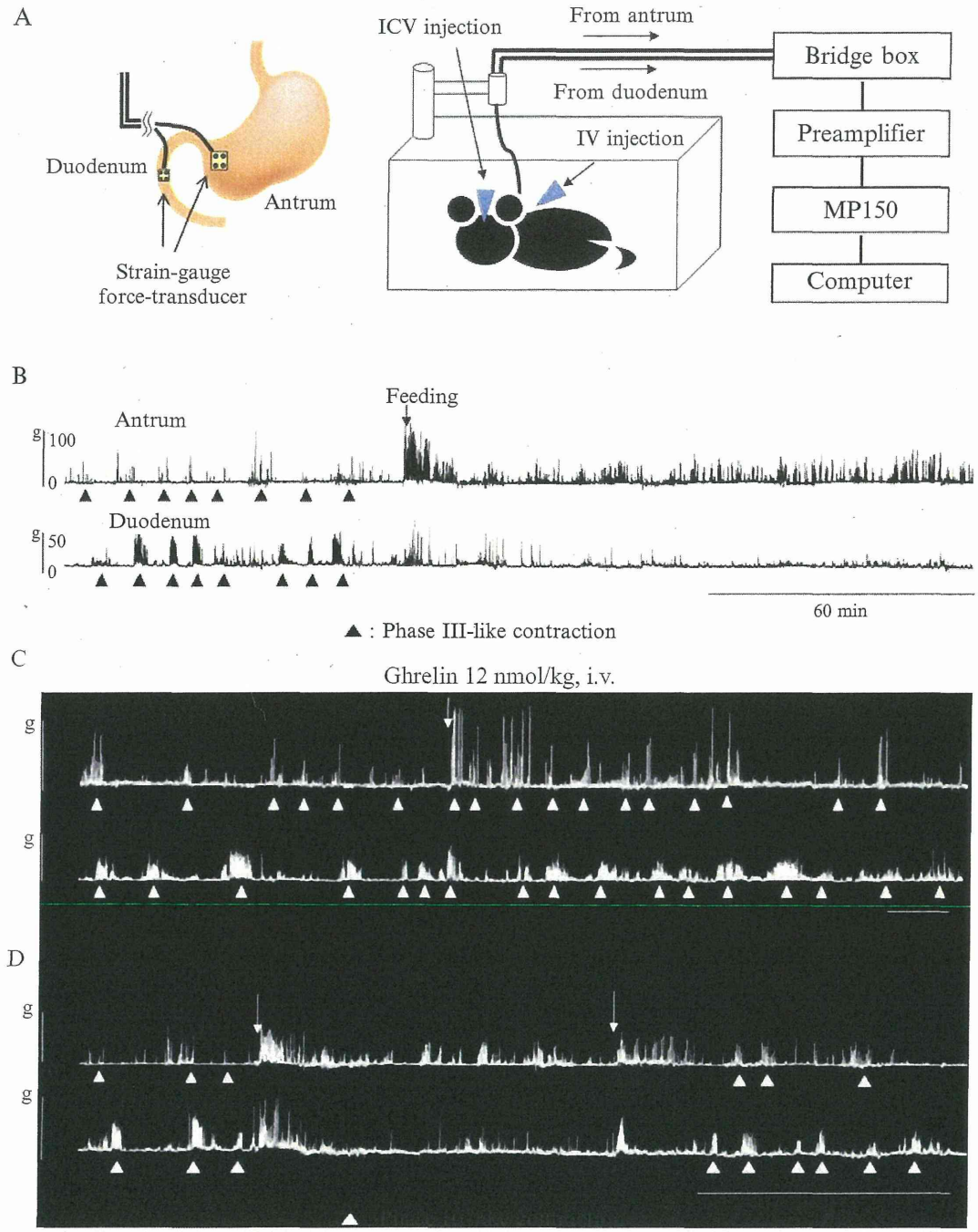

Figure 18.1 Method for strain-gauge force-transducer measurements of gastrointestinal motility in conscious rats. (A) Strain-gauge force transducers were placed on the serosal surface of the antrum and duodenum. The wires of the transducers were drawn out from the back of the neck and connected to a preamplifier via a bridge box. Data were recorded using an MP150. (B) The fasted patterns were replaced by the fed patterns in antrum and duodenum after feeding. (C) Intravenous administration of ghrelin to fasted rats immediately potentiated the frequency of phase III-like contractions in antrum and duodenum. (D) Intravenous administration of ghrelin eliminated the fed motor activities and induced phase III-like contractions in the antrum and duodenum of fed rats (from Fujitsuka et al., 2009).

2.1.1 Animal preparation involving the strain-gauge force-transducer method

1. Rats deprived of food overnight and weighing 200–250 g are anesthetized with intraperitoneal injections of pentobarbital sodium (50 mg/kg body weight).
2. After laparotomy, strain-gauge force transducers (F-08IS, Star Medical, Tokyo, Japan) are placed on the serosal surface of the antrum and duodenum.
3. The wires of the transducers are drawn out through a protective coil from the back of the neck via the subcutaneous part of the back.
4. Measurements are made with the animals in a free-moving condition system (Sugiyana-gen Co., LTD, Tokyo, Japan) in individual cages after a 5-day postoperative period for recovery.

2.1.2 Measurement of gastroduodenal motility

1. Rats are deprived of food but not water for 16 h before the experiment.
2. The strain-gauge force transducer placed in rats is connected to a preamplifier via a bridge box (Star Medical).
3. Data are recorded using an MP150 (BIOPAC Systems, Goleta, California).
4. The experiment is started when the fasted gastric contraction is stabilized, 2 h after the initial measurement.
5. The frequency of the fasted pattern is obtained from the average of the onset of phase III-like activities for each hour of the experiment.
6. The area under the wave (MI) per minute in the antrum and duodenum is measured and is shown as a percentage (%MI) relative to control data.

2.2. The manometric measurement of gastroduodenal motility in conscious rats and mice

Gastroduodenal motility in the physiological fed and fasted states of conscious rats has also been measured using a manometric method (Fig. 18.2A) (Fujimiya et al., 2000; Fujino et al., 2003). The frequency of phase III-like contractions in the antrum was 5.3 ± 0.5/h and that in the duodenum was 5.6 ± 0.8/h in fasted rats. This fasted pattern was disrupted and replaced by the fed pattern after feeding (Fig. 18.2B). The intravenous injection of ghrelin induced the fasted pattern in the duodenum when rats in the fed state were injected, increasing their %MI in the antrum (Fig. 18.2C). Recent advances in transgenic and knockout technologies have provided tools to investigate the pathogenesis of disease models, and these technologies have typically been applied to mice.

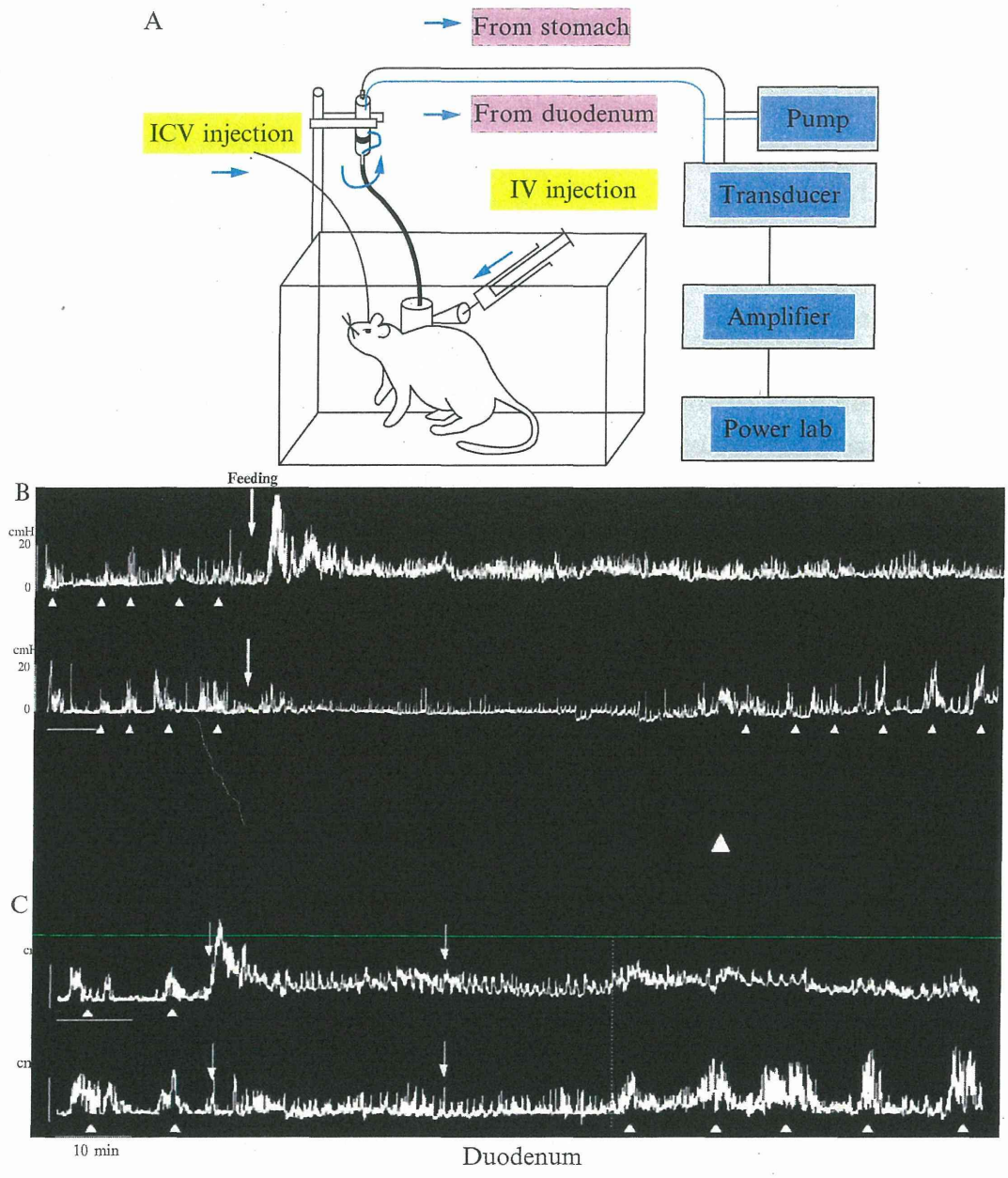

Figure 18.2 Method for manometric measurements of gastrointestinal motility in conscious rats. (A) Catheters for motility recordings are inserted into the antrum and duodenum, connected to the infusion swivel to allow free movement and then connected to a pressure transducer. The data are recorded and stored in a PowerLab. (B) Phase III-like contractions in fasted rats were disrupted and replaced by the fed pattern, which is irregular contraction of high frequency, after feeding. From Kihara et al. (2001). (C) Intravenous administration of ghrelin eliminated the fed motor pattern and produced a fasted motor pattern in duodenum (from Fujino et al., 2003).

Manometric methods allow dual monitoring of the motility of the stomach and duodenum in conscious mice (Tanaka et al., 2009). In fasted mice, the frequency of phase III-like contractions in the antrum was 7.8 ± 0.5/h and that in the duodenum was 6.6 ± 0.7/h. However, the frequency of phase

III-like contractions was lower in the antrum of neuropeptide Y (NPY) Y2 receptor knockout mice than in wild-type mice (Tanaka et al., 2009). The manometric method for the measurement of gastroduodenal motility in conscious rats and mice reported by Fujino et al. (2003) and Tanaka et al. (2009) is described below.

2.2.1 Animal preparation (manometric method)

1. Rats weighing 200–250 g or mice weighing 20–25 g are anesthetized by the intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight).
2. Two polyurethane tubes (3-Fr, 1 mm diameter for rats or ID 0.30 × OD 0.84 mm for mice) are used as a manometric catheter for the motility recordings.
3. One tube is inserted into the stomach through a small incision to the gastric body with the tip placed at the gastric antrum. The other is inserted through the duodenal wall, and the tip is placed 3 cm for rats or 7 mm for mice from the pylorus.
4. The tubes are fixed on the gastric wall and duodenal wall by purse-string sutures, which run subcutaneously to emerge at the top of the neck, and are then secured on the neck skin.
5. Animals are allowed to recover for 1 week before the experiments.

2.2.2 Measurement of gastroduodenal motility (Fig. 18.2A)

1. Mice are deprived of food but not water for 16 h before the experiment.
2. The manometric catheters from the stomach and duodenum are connected to the infusion swivel on a single-axis counter-weighted swivel mount to allow free movement and then connected to a pressure transducer.
3. The catheters are continuously infused with bubble-free saline or distilled water from an infusion pump at the rate of 1.5 ml/h for rats or 0.15 ml/h for mice.
4. The data are recorded and stored in a PowerLab.
5. The mice are placed in a black box (150 × 200 × 300 mm) with the top open.
6. Motor activity is analyzed as described in Section 2.1.2.

2.3. Measurement of gastroduodenal motility in conscious house musk shrews (*S. murinus*)

Ghrelin has a structural resemblance to motilin, and the ghrelin receptor exhibits a 50% identity with the motilin receptor (Asakawa et al., 2001). Ghrelin induces premature phase III contractions in the human stomach

(Tack et al., 2006). Motilin also induces phase III contractions through the cholinergic pathway in humans and dogs (Itoh et al., 1976; Luiking et al., 1998; Suzuki et al., 1998). Ghrelin and motilin are expected to have additive or synergistic effects on the induction of GI contraction. The motilin gene is inactivated in rodents, and mice and rats are therefore not suitable animals for the study of motilin–ghrelin interactions (He et al., 2010).

A recent report (Sakahara et al., 2010) has demonstrated that both physiological ghrelin and motilin are produced and stimulate gastric motility in the house musk shrew (laboratory name: suncus). Strain-gauge force transducers were implanted on the serosa of the gastric body and duodenum in the free-moving, conscious suncus. As a result, clear fasted contractions similar to those observed in humans (Vantrappen et al., 1977) and dogs (Szurszewski, 1969) were observed in the suncus stomach (gastric body). These coordinated contractions consist of three phases: phase I (a period of motor quiescence), phase II (a period of preceding irregular contractions), and phase III (a period of clustered potent contractions). These phases were clearly recognized at regular intervals (every 80–150 min). In addition, ghrelin and/or motilin stimulated suncus gastric motility (Sakahara et al., 2010). Suncus, a small laboratory animal, may be useful as an alternative to humans and dogs for studying the physiological relationships between ghrelin and motilin on GI motility.

3. THE GI MOTOR EFFECT OF GHRELIN MEDIATED BY THE GUT–BRAIN AXIS

3.1. The brain mechanism responsible for mediating GI motility

The fasted pattern in GI motility is disrupted and replaced by the fed pattern after feeding. Intracerebroventricular injection of NPY, a powerful orexigenic peptide in the brain, induces fasted motor activity in fed rats (Fujimiya et al., 2000). The frequency of phase III-like contractions was lower in the antrum of NPY-Y2 knockout mice than in wild-type mice (Tanaka et al., 2009). However, anorexigenic peptides such as the corticotrophin-releasing factor (CRF) (Bueno et al., 1986), urocortin (Kihara et al., 2001), and cholecystokinin (Rodriguez-Membrilla and Vergara, 1997) cause the disruption of fasted motor activity in animals. These findings may represent an integrated mechanism linking the feeding behavior and GI motor activity through the gut–brain axis.