The effects of intravenous injection of ghrelin were blocked by the immunoneutralization of NPY in the brain, suggesting that peripheral ghrelin induces fasted motor activity by activating the NPY neurons in the brain, probably through ghrelin receptors on vagal afferent neurons (Fujino et al., 2003). Various recent studies have demonstrated the brain mechanism responsible for mediating GI motility. Central and peripheral administration of des-acyl ghrelin has been shown to significantly decrease food intake in food-deprived mice and to decrease gastric emptying (Asakawa et al., 2005). Des-acyl ghrelin exerts inhibitory effects on antrum motility but not on duodenal motility in fasted animals (Chen et al., 2005). Obestatin exerts inhibitory effects on the motility of the antrum and duodenum in the fed state but not in the fasted state (Ataka et al., 2008). CRF receptors in the brain may mediate the actions of des-acyl ghrelin and obestatin. Central administration of nesfatin-1, which has been identified as a hypothalamic anorexigenic peptide, has been shown to decrease food intake and inhibit gastroduodenal motility in mice (Atsuchi et al., 2010). In the experiments that measure gastroduodenal motility, the peptide should be injected through a catheter to avoid the effect of handling stress. The methodology for catheter implantation in rats is described below.

3.1.1 Vessel catheter (Figs. 18.1A and 18.2A)

A vessel catheter (ID 0.36 × OD 0.84 mm, Eicom, Kyoto, Japan) is inserted into the right jugular vein in rats and also led out from the back of the neck. The catheter is filled with heparinized saline (100 units/ml) to avoid blood coagulation. The operation can be performed at the same time as the implantation of a strain-gauge force transducer.

3.1.2 Intracerebroventricular catheter (Figs. 18.1A and 18.2A)

1. The implantation of an intracerebroventricular catheter is performed 4 days before the implantation of a strain-gauge force transducer.
2. The anesthetized rats are placed in a stereotaxic apparatus and implanted with a guide cannula (25 gauge; Eicom, Kyoto, Japan), which reaches the right lateral ventricle.
3. The stereotaxic coordinates are 0.8 mm posterior to bregma, 1.4 mm right lateral to the midline, and 3.4 mm below the outer surface of the skull, when using a Kopf stereotaxic frame (Tujunga, CA, USA), with the incisor bar set at the horizontal plane passing through bregma and lambda.

4. The guide cannula is secured with dental cement anchored by two stainless steel screws that are fixed on the dorsal surface of the skull.
5. After surgery, a dummy cannula (Eicom) is inserted into each guide cannula and a screw cap (Eicom) is placed on the guide cannula to prevent blockade.
6. The correct placement of the intracerebroventricular catheter is verified by the administration of a dye (e.g., 0.05% cresyl violet) into the right lateral ventricle by the brain sections at the end of the experiments.

3.2. Ghrelin and GI disorders

Ghrelin and its receptor agonists possess strong prokinetic properties and therefore have the potential to serve in the treatment of diabetic, neurogenic, or idiopathic gastroparesis as well as for chemotherapy-associated dyspepsia; postoperative, septic, or postburn ileus; opiate-induced bowel dysfunction; and chronic idiopathic constipation (Sallam and Chen, 2010). Abnormalities in gastroduodenal motility are considered key players in the pathogenesis of upper-GI symptoms in certain disorders such as functional dyspepsia and gastroparesis (Suzuki et al., 2006). Zheng et al. (2009b) reported that acute restraint stress inhibits solid gastric emptying and abolishes gastric phase III-like contractions via central CRF in rats. During subsequent chronic stress, the impaired gastric phase III-like contractions were restored by an adaptation mechanism that involves the upregulation of ghrelin expression. Recent work has shown that the central serotonin (5-HT) 2c receptor pathway decreases the peripheral levels of ghrelin, resulting in a shift from fasted to fed-like motor activity. Intravenous administration of ghrelin was shown to replace fed with fasted motor activity in rats treated with fenfluramine, which stimulated 5-HT2cR signaling in the central nervous system. Rikkunshito is widely prescribed for patients exhibiting functional dyspepsia (Kusunoki et al., 2010; Suzuki et al., 2009). Oral administration of rikkunshito has been shown to reduce the incidence of anorexia and improve gastric emptying in animals through increased peripheral plasma ghrelin concentrations (Fujitsuka et al., 2009; Sadakane et al., 2011; Saegusa et al., 2011; Takeda et al., 2008; Yakabi et al., 2011), stimulated central ghrelin secretion (Yakabi et al., 2010), or increased hypothalamic ghrelin receptor activity (Takeda et al., 2010). Recent studies have demonstrated that oral administration of rikkunshito improves gastroduodenal dysmotility in a rat model of cancer anorexia–cachexia by the potentiation of ghrelin receptor signaling

(Fujitsuka et al., 2011). These findings suggest that stimulation of ghrelin signaling may be an attractive approach for the treatment of upper-GI motor dysfunction.

4. SUMMARY

Recent technical advances have permitted the measurement of GI motility in conscious small animals, including rats, mice, and house musk shrews (*S. murinus*). Transgenic and knockout mice are tools to investigate the pathogenesis of disease models. The suncus may be useful as an alternative to humans and dogs for studying the physiological relationships between ghrelin and motilin in the context of GI motility. Recent experiments on free-moving, conscious animal have demonstrated that ghrelin regulates physiological fasted motor activity in the antrum and duodenum. Intravenous injection of ghrelin increases the MI and the frequency of phase III-like contractions, both of which are mediated by hypothalamic NPY neuron activation though ghrelin receptors at the vagal afferent terminal.

Stress hormone and anorexigenic peptides cause the disruption of fasted motor activity through a brain–gut interaction, which is involved in the pathogenesis of upper-GI symptoms in disorders such as functional dyspepsia and gastroparesis. Ghrelin is a signal potentiator that promotes GI motility and could be a good therapeutic target for GI disorders.

ACKNOWLEDGMENTS

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. A. I. has received grant support from Tsumura & Co.

REFERENCES

Ariga, H., et al., 2007. Endogenous acyl ghrelin is involved in mediating spontaneous phase III-like contractions of the rat stomach. Neurogastroenterol. Motil. 19, 675–680.

Asakawa, A., et al., 2001. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 120, 337–345.

Asakawa, A., et al., 2005. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut 54, 18–24.

Ataka, K., et al., 2008. Obestatin inhibits motor activity in the antrum and duodenum in the fed state of conscious rats. Am. J. Physiol. Gastrointest. Liver Physiol. 294, G1210–G1218.

Atsuchi, K., et al., 2010. Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. Neuroreport 21, 1008–1011.

Bisschops, R., 2008. Ligand and electrically induced activation patterns in myenteric neuronal networks. Confocal calcium imaging as a bridge between basic and human physiology. Verh. K. Acad. Geneeskd. Belg. 70, 105–145.

Bueno, L., et al., 1986. Effects of corticotropin-releasing factor on plasma motilin and somatostatin levels and gastrointestinal motility in dogs. Gastroenterology 91, 884–889.
Chen, C.Y., et al., 2005. Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. Gastroenterology 129, 8–25.
Fujimiya, M., et al., 2000. Neuropeptide Y induces fasted pattern of duodenal motility via Y(2) receptors in conscious fed rats. Am. J. Physiol. Gastrointest. Liver Physiol. 278, G32–G38.
Fujino, K., et al., 2003. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. J. Physiol. 550, 227–240.
Fujitsuka, N., et al., 2009. Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT2c receptor and acyl ghrelin. Biol. Psychiatry 65, 748–759.
Fujitsuka, N., et al., 2011. Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival. Transl. Psychiatry 1, e23.
He, J., et al., 2010. Stepwise loss of motilin and its specific receptor genes in rodents. J. Mol. Endocrinol. 44, 37–44.
Inui, A., et al., 2004. Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. FASEB J. 18, 439–456.
Itoh, Z., et al., 1976. Motilin-induced mechanical activity in the canine alimentary tract. Scand. J. Gastroenterol. 39 (Suppl.), 93–110.
Kihara, N., et al., 2001. Effects of central and peripheral urocortin on fed and fasted gastroduodenal motor activity in conscious rats. Am. J. Physiol. Gastrointest. Liver Physiol. 280, G406–G419.
Kusunoki, H., et al., 2010. Efficacy of Rikkunshito, a traditional Japanese medicine (Kampo), in treating functional dyspepsia. Intern. Med. 49, 2195–2202.
Levin, F., et al., 2006. Ghrelin stimulates gastric emptying and hunger in normal-weight humans. J. Clin. Endocrinol. Metabol. 91, 3296–3302.
Luiking, Y.C., et al., 1998. Motilin induces gall bladder emptying and antral contractions in the fasted state in humans. Gut 42, 830–835.
Ohno, T., et al., 2010. The roles of motilin and ghrelin in gastrointestinal motility. Int. J. Pept. article ID 820794
Rodriguez-Membrilla, A., Vergara, P., 1997. Endogenous CCK disrupts the MMC pattern via capsaicin-sensitive vagal afferent fibers in the rat. Am. J. Physiol. 272, G100–G105.
Sadakane, C., et al., 2011. 10-Gingerol, a component of rikkunshito, improves cisplatin-induced anorexia by inhibiting acylated ghrelin degradation. Biochem. Biophys. Res. Commun. 412, 506–511.
Saegusa, Y., et al., 2011. Decreased plasma ghrelin contributes to anorexia following novelty stress. Am. J. Physiol. Endocrinol. Metab. 301, E685–E696.
Sakahara, S., et al., 2010. Physiological characteristics of gastric contractions and circadian gastric motility in the free-moving conscious house musk shrew (Suncus murinus). Am. J. Physiol. Regul. Integr. Comp. Physiol. 299, R1106–R1113.
Sallam, H.S., Chen, J.D., 2010. The prokinetic face of ghrelin. Int. J. Pept. article ID 493614
Suzuki, H., et al., 1998. Motilin controls cyclic release of insulin through vagal cholinergic muscarinic pathways in fasted dogs. Am. J. Physiol. 274, G87–G95.
Suzuki, H., et al., 2006. Therapeutic strategies for functional dyspepsia and the introduction of the Rome III classification. J. Gastroenterol. 41, 513–523.
Suzuki, H., et al., 2009. Japanese herbal medicine in functional gastrointestinal disorders. Neurogastroenterol. Motil. 21, 688–696.
Szurszewski, J.H., 1969. A migrating electric complex of canine small intestine. Am. J. Physiol. 217, 1757–1763.
Tack, J., et al., 2006. Influence of ghrelin on interdigestive gastrointestinal motility in humans. Gut 55, 327–333.

Takeda, H., et al., 2008. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. Gastroenterology 134, 2004–2013.
Takeda, H., et al., 2010. Rikkunshito ameliorates the aging-associated decrease in ghrelin receptor reactivity via phosphodiesterase III inhibition. Endocrinology 151, 244–252.
Tanaka, R., et al., 2009. New method of manometric measurement of gastroduodenal motility in conscious mice: effects of ghrelin and Y2 depletion. Am. J. Physiol. Gastrointest. Liver Physiol. 297, G1028–G1034.
Vantrappen, G., et al., 1977. The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. J. Clin. Invest. 59, 1158–1166.
Yakabi, K., et al., 2010. Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction. Regul. Pept. 161, 97–105.
Yakabi, K., et al., 2011. Urocortin 1 reduces food intake and ghrelin secretion via CRF(2) receptors. Am. J. Physiol. Endocrinol. Metab. 301, E72–E82.
Zheng, J., et al., 2009a. Ghrelin regulates gastric phase III-like contractions in freely moving conscious mice. Neurogastroenterol. Motil. 21, 78–84.
Zheng, J., et al., 2009b. Effects of repeated restraint stress on gastric motility in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R1358–R1365.

Editorial

A New Horizon of Herbal Medicines in Anorexia-Cachexia Syndrome

The role of complementary and alternative medicine (CAM) continues to evolve in the daily lifestyle and treatment regimens of patients such as cancer. More than half of the cancer patients have used some form of CAM treatment during their cancer therapy in USA, and the situation is similar in other nations such as Europe and Japan. CAM use may be inclusive of holistic spiritual practice and physical exercise, as well as vitamins and herbal medicines for enhanced tumoricidal activity or reduction in treatment-related adverse events. Herbal medicine has been practiced for a long time in China, Korea, Japan, and other countries to achieve its key goal of restoring the balance of energy in the body.

Many effective chemotherapeutic agents for cancer are burdened by toxicities that can reduce patient quality of life or hinder their effective use. Attempts to minimize the toxicity by using isolated compounds have been unsatisfactory. Herbal medicines, composed of multiple biologically active compounds, are widely used to help improve such conditions. Recent studies have shown that the herbal medicines such as rikkunshito improve nausea, appetite loss and cachexia associated with cancer or cancer chemotherapy which worsens QOL and life expectancy of the patients. The mechanism involves an enhancement of signaling by ghrelin [1, 2] which was discovered in 1999 as an appetite-stimulating peptide from the stomach. It has a rivaling action to leptin, an afferent signal from fat tissue which informs the brain the size of body adiposity [3]. Currently, ghrelin agonists and antagonists are being developed and tested for treatment of anorexia/cachexia and obesity, respectively.

Although herbal medicines have not been fully accepted by mainstream medicine because of the complex nature of the formulae, the stringent quality control of Japanese herbal medicine and reproducibility of preclinical findings, together with few adverse events, have made herbal medicines more and more attractive for the management on intractable diseases such as cancer. The multi-component herbal medicines capable of targeting multiple sites could be useful for future drug discovery. Mechanistic studies and identification of active compounds could lead to new discoveries in biological and biomedical sciences.

This review series cover the translational aspects of herbal medicine on cancer treatment, particularly for cancer anorexia-cachexia syndrome (Fig. **1**). A focus will be put on rikkunshito and its active components that are able to potentiate ghrelin signaling [4] and mitigate the anorexia-cachexia syndrome. The review would provide a new horizon of herbal medicine from scientific point of view and be a basis for further development of CAM for patients with cancer and other intractable diseases.

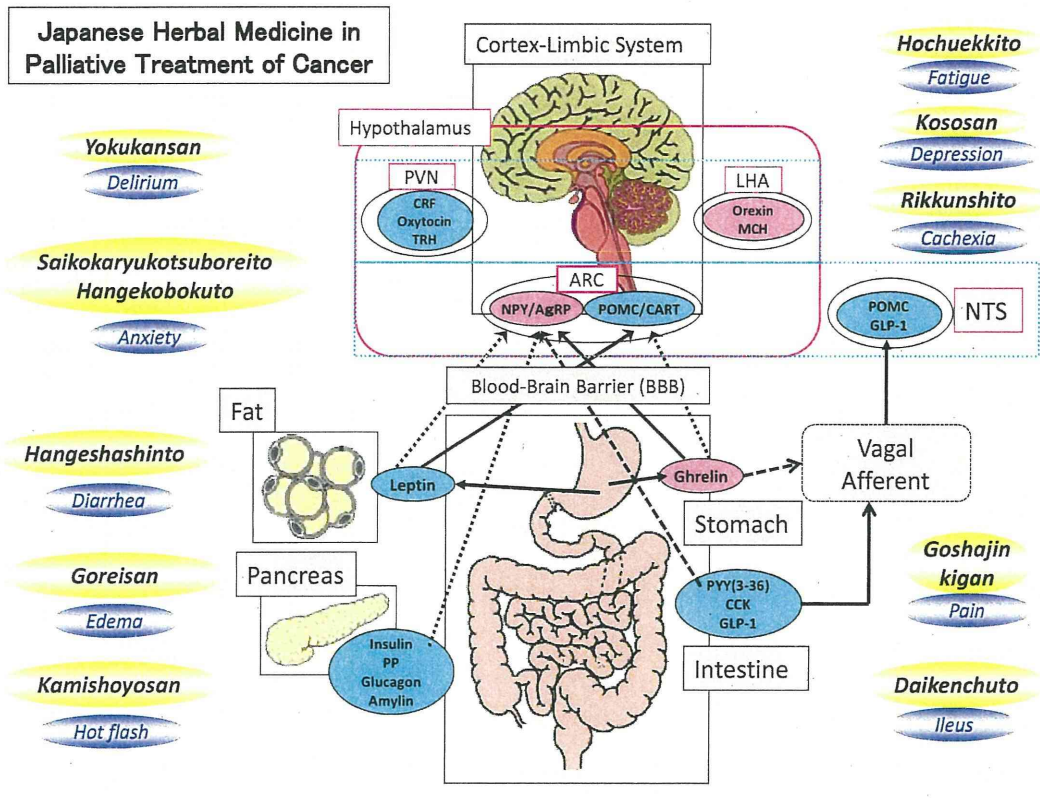

Fig. (1). Shown are the examples of Japanese herbal medicine in the palliative treatment of cancer. The quality control of herbal medicines is performed by 3 dimensional HPLC analysis that roughly estimates the main components of the crude drugs. The herbal medicines depicted are used to improve the cancer associated conditions such as anorexia-cachexia, depression, fatigue, anxiety, delirium, ileus, pain, edema, diarrhea and hot flash based on the scientific evidence in animal experiments and human studies. Brain-gut peptides are deeply involved in the actions of several herbal medicines: ghrelin in rikkunshito, adrenomedullin in daikenchuto, orexin in kososan, and CRF and opioid system in saikokaryukotuboreito and goshajinkigan. Solid lines show stimulation and dotted lines inhibition. See Perspectives in the last chapter of this special issue for details.

Abbreviations: Neuropeptide Y (NPY); pancreatic polypeptide (PP); melanin-concentrating hormone (MCH); agouti-related peptide (AgRP); corticotropin-releasing factor (CRF); glucagon-like peptide I (GLP-I); cocaine- and amphetamine-related transcript (CART); proopiomelanocortin (POMC); arcuate nucleus (ARC); paraventricular nucleus (PVN); cholecystokinin (CCK); lateral hypothalamic area (LHA); nucleus tractus solitarius (NTS); thyrotropin-releasing hormone (TRH)

REFERENCES

[1] Takeda H, Sadakane C, Hattori T, *et al.* Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. Gastroenterology 2008; 134: 2004-13.

[2] Fujitsuka N, Asakawa A, Hayashi M, *et al.* Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT2c receptor and acyl ghrelin. Biological Psychiatry 2009; 65: 748-59.

[3] Chen, C.-Y., Inui, A. et al. Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev 2009: 61: 430–481.

[4] Fujitsuka N, Asakawa A, Uezono Y, *et al.* Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival. Translational Psychiatry 2011; 1: e23-10.

Akio Inui, MD, PhD

Department of Psychosomatic Internal Medicine,
Kagoshima University Graduate School of
Medical and Dental Sciences,
Kagoshima, 890-8520,
Japan
E-mail: inui@m.kufm.kagoshima-u.ac.jp

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2013;43(7)695–705
doi:10.1093/jjco/hyt075
Advance Access Publication 4 June 2013

Cancer Cachexia Pathophysiology and Translational Aspect of Herbal Medicine

Hajime Suzuki[1,2], Akihiro Asakawa[1], Haruka Amitani[1], Naoki Fujitsuka[1,3], Norifumi Nakamura[2] and Akio Inui[1,*]

[1]Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, [2]Department of Oral and Maxillofacial Surgery, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima and [3]TSUMURA Research Laboratories, Tsumura & Co., Ibaraki 300-1192, Japan

*For reprints and all correspondence: Akio Inui, Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima, 890-8520, Japan. E-mail: inui@m.kufm.kagoshima-u.ac.jp

Received October 17, 2012; accepted April 21, 2013

About half of all cancer patients show a syndrome of cachexia, characterized by anorexia and loss of adipose tissue and skeletal muscle mass. Numerous cytokines have been postulated to play a role in the etiology of cancer cachexia. Cytokines can elicit effects that mimic leptin signaling and suppress orexigenic ghrelin and neuropeptide Y signaling, inducing sustained anorexia and cachexia not accompanied by the usual compensatory response. Furthermore, cytokines have been implicated in the induction of cancer-related muscle wasting. In particular, tumor necrosis factor-alpha, interleukin-1, interleukin-6 and interferon-gamma have been implicated in the induction of cancer-related muscle wasting. Cytokine-induced skeletal muscle wasting is probably a multifactorial process, which involves a depression in protein synthesis, an increase in protein degradation or a combination of both. Cancer patients suffer from the reduction in physical function, tolerance to anti-cancer therapy and survival, while many effective chemotherapeutic agents for cancer are burdened by toxicities that can reduce patient's quality of life or hinder their effective use. Herbal medicines have been widely used to help improve such conditions. Recent studies have shown that herbal medicines such as *rikkunshito* enhance ghrelin signaling and consequently improve nausea, appetite loss and cachexia associated with cancer or cancer chemotherapy, which worsens the quality of life and life expectancy of the patients. The multicomponent herbal medicines capable of targeting multiple sites could be useful for future drug discovery. Mechanistic studies and identification of active compounds could lead to new discoveries in biological and biomedical sciences.

Key words: appetite loss – muscle wasting – cytokine – ghrelin – palliative cancer treatment – herbal medicine

INTRODUCTION

Cancer patients suffer from weight loss and appetite loss, as well as from the reduction in physical function, tolerance to anti-cancer therapy and survival that are related to cachexia in advanced cancer (1). Cachexia is a debilitating state of involuntary weight loss complicating malignant, infectious and inflammatory diseases and contributing significantly to mortality (2). The word 'cachexia' is derived from the Greek words 'kakos' meaning 'bad' and 'hexis' meaning 'condition' (3). Anorexia, involuntary weight loss, tissue wasting, poor performance and ultimately death characterize cancer cachexia—a condition of advanced protein calorie malnutrition (2–7). Referred to as 'the cancer anorexia–cachexia syndrome', anorexia, or loss of compensatory increase in

feeding, is a major contributor to the development of cachexia (8). About half of all cancer patients show a syndrome of cachexia, characterized by anorexia and loss of adipose tissue and skeletal muscle mass (2). In general, while patients with hematological malignancies and breast cancer seldom have substantial weight loss, most other solid tumors are associated with a higher frequency of cachexia (8). Weight loss and problems with nutrition may also be a significant emotional burden, as nutrition and nutritional status have a central position in the concept of health and wellbeing for many patients and care givers, and weight loss and inadequate nutritional intake can lead to anxiety and hopelessness (1). In contrast to these needs, cachexia often is overlooked or not assessed or treated adequately, as it is considered to be unavoidably linked with disease progression (1). Cachexia represents a significant unmet need (1).

PATHOPHYSIOLOGY

Neuropeptidergic Cascade Downstream of Leptin Signaling

Leptin is an afferent signal from the periphery to the brain that regulates adipose tissue mass (9–11). The level of leptin is positively correlated with body fat mass, and dynamic changes in plasma leptin concentrations in either direction activate the efferent energy regulation pathways (9,12). Leptin reduces appetite and increases energy expenditure and evidently elicits these effects via the central nervous system (9,12). This is achieved by hypothalamic neuropeptides downstream of leptin that regulate food intake and energy expenditure. A loss of body fat (starvation) leads to a decrease in leptin, which in turn leads to a state of positive energy balance, wherein food intake exceeds energy expenditure. This compensatory response is mediated by the increased production, release, and/or action of ghrelin, neuropeptide Y (NPY) and other orexigenic neuropeptides, as well as decreased activity of anorexigenic neuropeptides such as corticotropin-releasing factor (CRF) and melanocortin (Fig. 1A). Thus, if a disease process was to produce factors that induce or mimic the hypothalamic effect of excess negative feedback signaling from leptin, the expected outcome would be sustained anorexia and weight loss that is not accompanied by the usual compensatory response (2).

In tumor-bearing states, cachectic factors such as cytokines elicit effects on energy homeostasis that mimic leptin in some respects and suppress orexigenic ghrelin-NPY signaling. Consequently, the increases and decreases in hypothalamic actions caused by these mediators induce anorexia and unopposed weight loss, respectively (Fig. 1B). This could be accomplished through persistent inhibition of the

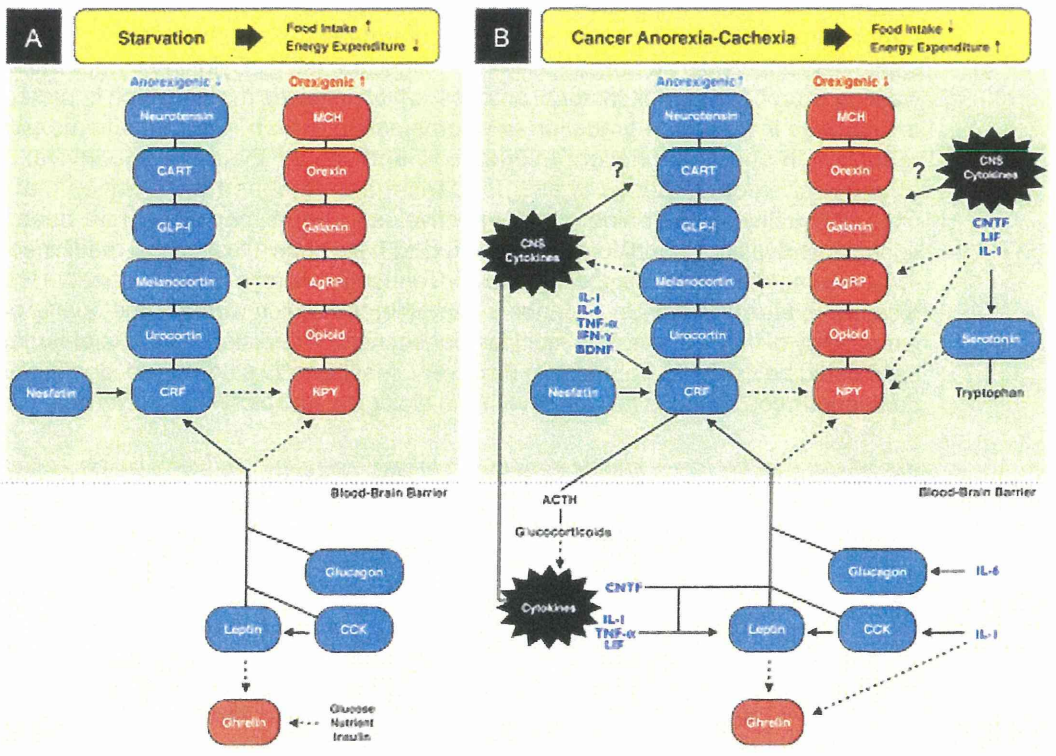

Figure 1. A simplified model of the hypothalamic neuropeptide circuitry in response to starvation (A) and cancer anorexia–cachexia (B) Source: (2) with modification. Full line arrows indicate the activation of the process, and broken line arrows indicate the inhibition of the process. AgRP = agouti-related peptide. MCH = melanin-concentrating hormone. CART = cocaine- and amphetamine-related transcript. GLP-I = glucagon-like peptide-I. CCK = cholecystokinin. IL-1 = interleukin-1. IL-6 = interleukin-6. TNF-α = tumor necrosis factor-alpha. IFN-γ = interferon-gamma. CNTF = ciliary neurotrophic factor. LIF = leukemia inhibitory factor. ACTH = adrenocorticotropic hormone.

ghrelin-NPY orexigenic network and stimulation of anorexigenic neuropeptides, although the hypothalamic pathways participating in this response remain to be determined. Serotonin may also play a role in the development of cancer anorexia. Increased levels of plasma and brain tryptophan, the precursor of serotonin and IL-1 may underlie the increased serotonergic activity seen in the cancer anorexia–cachexia syndrome.

Nesfatin-1, a new anorectic peptide localized to the paraventricular nucleus (PVN), is stimulated by stressors. Intracerebroventricular administration of nesfatin-1 activates 5HT neurons, CRF neurons and the hypothalamic-pituitary-adrenal axis, and nesfatin-1 activates isolated CRF neurons (13). It has recently been shown that plasma nesfatin-1 levels are altered in lung cancer patients with anorexia–cachexia. Hence, nesfatin-1 appears to be involved in cancer anorexia–cachexia. Novel bioactive peptides such as neuropeptide W and neuroendocrine regulatory peptide have also recently been identified; however, the role of these proteins in cancer anorexia–cachexia remains to be determined.

Cytokine Actions within the Regulatory Feedback Loop

Numerous cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), have been postulated to play a role in the etiology of cancer cachexia (3,14–18). It is not certain whether the cytokine production is primarily from tumor or host inflammatory cells. It has been hypothesized that either tumor cell production of pro-inflammatory cytokines or the host inflammatory cell response to tumor cells is the source of the acute phase protein response seen in many malignancies and in cachexia (19).

Cytokines are protein molecules released by lymphocytes and/or monocyte macrophages (2). They may be released into the circulation and transported to the brain through the blood–brain barrier (BBB) and circumventricular organs (leaky areas in the BBB), as is the case for IL-6 (17,18,20–23). Peripheral cytokines may influence the brain via neural pathways or second messengers such as nitric oxide (NO) and prostanoids (2). Cytokines are also produced by neurons and glial cells within the brain, partly in response to peripheral cytokines (17,18,20–23). Although the site of synthesis of cytokines within the brain is dependent on the nature of the stimulus, systemic disease seems to predominantly influence the expression in the hypothalamus, the area with the highest densities of receptors for most cytokines that have been observed (22).

High serum levels of TNF-α, IL-6 and IL-1 have been found in some, but not all, cancer patients, and the levels of these cytokines seem to correlate with the progression of some tumors (24–26). Chronic administration of these cytokines, either alone or in combination, is capable of reducing food intake and reproducing different features of the cancer anorexia–cachexia syndrome (3,24–27). The role of TNF-α in mediating cancer-associated anorexia is supported by evidence that intraperitoneal injection of a recombinant human soluble TNF receptor antagonist improves anorexia in tumor-bearing animals (28). In humans, IL-1 appears to play a significant role in mediating anorexia–cachexia, as megestrol acetate has been shown to exert its effects via reduced expression of IL-1 by mononuclear cells beyond its influence on hypothalamic neuropeptide Y (NPY) concentrations, which shows an orexigenic effect (29). Interestingly, anorexigenic neurons, such as proopiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART) neurons, in the arcuate nucleus (ARC) of the hypothalamus express the type 1 IL-1 receptor, and intracerebroventricular injection of IL-1 increases the frequency of action potentials of POMC/CART neurons and stimulates the release of alpha-melanocyte-stimulating hormone (α-MSH), which shows an anorexigenic effect as well (30).

TNF-α, IL-1, IL-6 and IFN-γ have been implicated in the induction of cancer-related muscle wasting (31). There is growing evidence that the accelerated muscle proteolysis seen during malignant tumor growth is mediated by the activation of the non-lysosomal adenosine triphosphate-dependent (ATP-dependent) ubiquitin proteasome pathway (32,33). In addition, inflammatory cytokines influence the expression of functionally relevant enzymes in cardiac cachexia (31). It has been demonstrated that TNF-α, IFN-γ and IL-1β, which are known to be increased in cachectic patients, are potent activators of inducible NO synthase (iNOS) expression (31), which in turn produces toxic levels of NO high enough to inhibit the key enzymes of oxidative phosphorylation (31). It has also been shown *in vitro* that NO is able to impair the contractile performance of skeletal muscle (34).

More direct evidence of cytokine involvement comes from experiments in which specific neutralization of cytokines can relieve anorexia and cachexia in experimental animal models (3,25,26,35). Examples of antibodies that have been shown to successfully relieve anorexia and cachexia when administered include the anti-TNF-α, anti-IL-6, anti-IL-1 and anti-IFN-γ antibodies, although no single antibody has been proven to reverse all of the features of wasting seen in cancer cachexia (25). These studies revealed that cachexia can rarely be attributed to any one cytokine but rather is associated with a set of cytokines and other cachectic factors that work in concert (2). Current new trends include the use of an anti-IL-6 humanized monoclonal antibody, which appears to inhibit cancer cachexia in murine models (36). The therapeutic impact of which on cancer-related anorexia and cachexia may be of clinical significance in cancer patients (36).

The problem with ascribing specific tissue responses to individual cytokines is that considerable overlap and redundancy exist in the cytokine network (16,20–23). Administration of either TNF-α or IL-1 will induce the synthesis of a variety of other proinflammatory cytokines, such as IL-6 (2). Thus, studies that use pharmacological administration of recombinant cytokines may not discriminate between biological responses induced directly by the administered cytokine and those induced secondarily by other stimulated cytokines (2). Systemic disease such as cancer and inflammation may elicit a cytokine cascade in which several cytokines are induced simultaneously (16).