Hypothalamic Neuropeptides and Appetite Response in Anorexia-Cachexia Animal

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CACHEXIA, characterized by anorexia and marked weight loss, is a complex syndrome in patients with inflammatory disease and cancer. In particular, the pathogenesis of cancer cachexia is a multifactor syndrome that includes reduced food intake, alteration in energy and substrate metabolism in the host and accelerated fat and muscle loss [1]. As anorexia-cachexia syndrome compromises the quality of life and contributes to mortality in patients, it is important to examine the essential mechanisms of the syndrome in order to develop effective therapeutics even though anorexia-cachexia syndrome may be caused by multiple factors.

In this review, we focus on the hypothalamic neuropeptide gene expression in an anorexia-cachexia animal model because neuropeptides in the hypothalamus may be the key molecules that regulate the appetite and feeding behavior in cancer anorexia-cachexia syndrome [2] as well as in normal conditions [3, 4]. Quantitative changes in neuropeptides gene expressions in the hypothalamus and the relevance of their changes in abnormal nutritional conditions (e.g., negative energy balance) are reviewed and discussed by a comparison of three different types of anorexia and anorexia-cachexia animal models; dehydration-induced anorexia, bacterial lipopolysaccharide (LPS)-induced anorexia, and cancer-induced anorexia-cachexia syndrome.

Hypothalamic neuropeptides related to appetite and feeding behaviors

It is well established that appetite and feeding behaviors are primarily controlled by a feeding center, the lateral hypothalamic area (LHA), and a satiety center, the ventromedial hypothalamic nucleus (VMH), in the hypothalamus [5]. Recently, neural networks and chemical mediators among the hypothalamic nuclei—not only the LHA and the VMH but also the arcuate nucleus (Arc) and the paraventricular nucleus (PVN)—related to feeding regulation have been studied by many investigators. Various kinds of neuropeptides as well as classic neurotransmitters such as dopamine and serotonin act as feeding regulatory factors in the neural networks at these sites [6].

For example, centrally administered neuropeptide Y (NPY) strongly stimulates feeding in mice and rats [7]. Many studies have demonstrated that the expression of the NPY gene was markedly increased in the neurons of the Arc after food deprivation (starvation). This type of peptide is called an “orexigenic” neuropeptide. Orexins/hypocretins, which are produced in the neurons located in the LHA [5], are also potent orexigenic neuropeptides. On the other hand, proopiomelanocortin (POMC), which encodes α melanocyte-stimulating...
hormone (αMSH) in the Arc and corticotrophin-releasing hormone (CRH) in the PVN function as the inhibitory system for feeding [8]. These neuropeptides are referred to as “anorexigenic” neuropeptides. Recently, many orexigenic and anorexigenic neuropeptides that regulate appetite and body weight have been discovered in the hypothalamus [3, 4, 6].

In general, orexigenic neuropeptides in the hypothalamus such as NPY and orexins/hypocretins stimulate feeding. Starvation (negative energy balance) up-regulates the expression of the orexigenic neuropeptide genes, and anorexigenic neuropeptides in the hypothalamus such as αMSH suppress feeding; starvation down-regulates the expression of the anorexigenic neuropeptide genes.

Dehydration-induced anorexia

Anorexia, the loss of appetite for food, is caused by various different mechanisms. Because the pathological model of anorexia may involve multiple factors, a simple method of developing anorexia in animal models is required in order to examine the neural and molecular mechanisms responsible for anorexia. With this physiological aspect in mind, we took notice of reports on dehydration-induced anorexia animal models [9–11].

Watts and his colleagues showed that rats exhibit profound anorexia from dehydration when hypertonic saline (2.5% NaCl) instead of drinking water was given to rats for 5 days [12, 13]. This model is advantageous in the examination of the neural network and molecules associated with dehydration-induced anorexia because slowly developed anorexia after dehydration is rapidly reversed once access to drinking water is restored.

Changes in the expression of the neuropeptides genes in the hypothalamus were examined in dehydration-induced anorexia animals [11–13], and in dehydrated rats, NPY mRNAs in the Arc significantly increased compared to euhydrated rats [12]. In addition, POMC and neurotensin/neuromedin N (NT/N) mRNAs in the Arc and CRH mRNA in the PVN of dehydrated rats were marked decreased in comparison with those in euhydrated rats [12]. These changes are reasonable as a result of the negative energy balance because drinking hypertonic saline caused anorexia with dehydration in the rats. It has also been demonstrated that CRH and NT/N mRNAs in the LHA were increased in dehydration-induced anorectic rats and CRH mRNA in the LHA strongly correlated with the intensity of anorexia. These results suggest that the dehydration-induced elevation of CRH and NT/N production in the LHA act upon the PVN and suppress feeding in dehydrated rats [11–13].

These studies raise the hypothesis that dehydration primarily causes up-regulation of the gene expression of anorexigenic neuropeptides in the hypothalamus which induces anorexia, and then anorexia-induced starvation (negative energy balance) up-regulates the gene expression of the orexigenic neuropeptides and reduces the gene expression of the anorexigenic neuropeptides (Fig. 1). The reason why dehydration causes up-regulation of the gene expression of anorexigenic neuropeptides in a feeding center and the possible involvement of peripheral humoral factors such as leptin and ghrelin on dehydration-induced anorexia should be clarified by further study.

LPS-induced anorexia

A pathological anorectic animal model as well as a physiological model such as dehydration-induced anorexia is also important for the examination of the mechanisms of anorexia. Anorexia and weight loss are common symptoms seen both in patients with inflammatory disease and in experimental animal models with inflammation.

Peripheral administration of bacterial LPS (a component of the outer membrane of gram-negative bacteria) to animals is often used to develop an experimental inflammatory animal model. Sickness syndromes such as fever, activation of the hypothalamo-pituitary adrenal axis, and anorexia occurs in response to peripheral administration of LPS [14–16]. It is interesting that LPS-induced anorexia is not related
to the fever induced by LPS [17, 18]. Although vagal afferents is known to be involved in the behavioral effects induced by peripheral administration of LPS, vagotomy does not attenuate the anorectic response to peripheral administration of LPS [19]. It has been postulated that the central effects of peripheral LPS may be mediated by cytokines, LPS receptors on the surface of the cerebral endothelial cells of the blood-brain barrier, and humoral mediators such as prostaglandins [20, 21].

Several studies have examined the changes in the gene expressions of orexigenic and anorexigenic neuropeptides in the hypothalamus of LPS-treated animals [22–24]. Sergeyev et al. examined the changes of neuropeptides-POMC, NPY, galanin, MCH and cocaine- and amphetamine-regulated transcripts (CART)-in the hypothalamus after intraperitoneal (ip) injection of LPS in rats using in situ hybridization histotechnology [23]. They confirmed a reduction of food intake 4 hours after ip injection of LPS. In the hypothalamus of the LPS-treated rats POMC and CART mRNAs in the Arc showed an increase in comparison with the control. On the other hand, MCH, CART and galanin mRNAs in the LHA decreased in the LPS-treated rats. Thus, suggesting that up-regulation of anorexigenic neuropeptides (POMC and CART) in the Arc and down-regulation of orexigenic neuropeptides (MCH and galanin) in the LHA may have been the cause of the reduction of food intake after ip administration of LPS [23]. In LPS-treated rats, αMSH derived from POMC has been suggested to mediate LPS-induced anorexia [25]. They postulated that NPY mRNA in the Arc did not change in either the LPS-treated or control rats because the time interval (4 hours after ip administration of LPS) may be too short for transcriptional change to be detected. In mice, NPY and agouti-related protein (AgRP) mRNAs in the hypothalamus were significantly increased at 6 hours after LPS injection [24].

These results raised the hypothesis that peripheral administration of LPS indirectly causes up-regulation of the gene expression of anorexigenic neuropeptides in the hypothalamus and down-regulation of the gene expression of orexigenic neuropeptides in the hypothalamus (Fig. 2). It is natural that these changes in hypothalamic neuropeptide gene expression induce anorexia. The up-regulation of the orexigenic neuropeptide genes in the hypothalamus in mice at 6 hours after LPS injection may be a result of anorexia induced by LPS. Peripheral administration of LPS induces production and secretion of prostaglandins and various cytokines. Thus, further study is necessary to examine the crucial factors involved in the pathogenesis of LPS-induced anorexia.

**Cancer anorexia-cachexia syndrome**

Anorexia-cachexia syndrome is found in a large number of patients in the advanced stages of cancer [26, 27]. Several metabolic and behavioral abnormalities such as appetite loss, loss of skeletal muscle and fat, general fatigue, and psychological distress are observed in this syndrome. It is difficult to find a common factor that causes cancer anorexia-cachexia syndrome because multiple factors may be involved in the etiology of this syndrome [1, 2, 28–31].

In order to reveal the etiology of cancer anorexia-cachexia syndrome, it is important to investigate changes in orexigenic and anorexigenic neuropeptides in the hypothalamus in cancer-associated anorexia-cachexia animal models. Although multiple factors are involved in cancer anorexia-cachexia syndrome, the changes in these neuropeptides in the hypothalamus may elucidate the mechanisms in cancer anorexia-cachexia syndrome. The involvement of cytokines and hypothalamic neuropeptides in cancer anorexia-cachexia syndrome has been well reported [1, 2, 32, 33, 34]. It has been demonstrated that cachectic factors such as cytokines mimic the effects of leptin on the hypothalamus and induce anorexia in cancer anorexia-cachexia syndrome (Fig. 3).

Leptin is the product of the *ob* gene. Starvation suppresses the expression of the *ob* gene in adipocytes and decreases plasma leptin levels [35, 36]. Leptin deficiency due to mutation of the *ob* gene (*ob/ob* mouse) and leptin resistance due to mutation of the leptin re-
ceptor (db/db mouse and fa/fa rat) cause severe obesity [35, 37, 38]. NPY mRNA in the Arc of ob/ob mouse, db/db mouse, and fa/fa rat were markedly increased in comparison with controls [39, 40].

Several studies have demonstrated changes in the gene expression of hypothalamic orexigenic and anorexigenic neuropeptides in cachexia animal models bearing cancer cells such as the following.

Orexigenic neuropeptides (NPY, AgRP, orexins, MCH) in anorexia-cachexia syndrome

Plata-Salaman et al. reported that NPY mRNA and POMC mRNA levels in the hypothalamus were not significantly different between pair-fed normal rats and anorectic rats bearing prostate adenocarcinoma tumor cells [30]. Nara-ashizawa et al. also demonstrated that NPY mRNA in the Arc was up-regulated in cachectic nude mice bearing human tumor cells (SEKI melanoma cells) [41]. They also showed that there is no significant difference in MCH and orexin mRNA levels between the SEKI and control mice [41]. Jensen et al. demonstrated that NPY mRNA levels in the Arc were markedly increased in anorexic rats bearing glucagonoma (MSL-G-AN) [42]. Chance et al. demonstrated that NPY mRNA levels in the medial hypothalamus were significantly elevated in anorectic tumor-bearing (methylcholanthrene-induced sarcoma) rats, and that CRH mRNA in the medial hypothalamus tend to be reduced in anorectic tumor-bearing rats and paired-fed rats [43]. Chen et al. demonstrated that hypothalamic NPY mRNA was significantly raised in cachetic MAC16 (colonic adenocarcinoma)-bearing mice and pair-fed mice [44].

Although it is difficult to explain why the orexigenic neuropeptide NPY up-regulates in the hypothalamus of cachectic tumor-bearing animals, one explanation might be that downstream of NPY mRNA, functions such as protein synthesis, transport, secretion and its receptors are disturbed by humoral factors such as cytokines and tumor-derived factors [45]. Another explanation for some cases is that hypothalamic NPY mRNA response is less sensitive in cachectic mice bearing human tumor cells (SEKI, NAGAI, and G361) in comparison with pair-fed normal rats, and that MCH and orexin mRNA in the hypothalamus follows a pattern similar to NPY [46].

Anorexigenic neuropeptides (aMSH, CART, CRH) in anorexia-cachexia syndrome

Plata-Salaman et al. reported that POMC mRNA levels in the hypothalamus were not significantly different between pair-fed normal rats and anorectic rats bearing prostate adenocarcinoma tumor cells [30]. Chance et al. demonstrated that POMC mRNA was not changed in cachectic tumor-bearing rats [46]. Nara-ashizawa et al. demonstrated that CRH mRNA in the PVN was up-regulated in cachectic tumor-bearing mice [41]. Jensen et al. demonstrated that hypothalamic CART is highly expressed in anorectic rat glucagonoma [48].

As we mentioned above, it may be difficult to come to a conclusion on the relationship between the hypothalamic neuropeptides and appetite loss in cachectic animal models because previous reports are not concordant. One of reasons may be based on the different tumor cells used for tumor-bearing models and the different methods of evaluation such as Northern blot analysis, RNA protection assay, and in situ hybridization histochemistry. In addition, the studies have not examined all of the orexigenic/anorexigenic neuropeptides in the hypothalamus. Another weak point is that the cachexia animal models are not recovered by therapeutic treatment, thus it is difficult to compare the changes of the neuropeptides in the hypothalamus at all stages- pre-cachetic, cachetic, and recovery from the cachexia.

To resolve these issues we selected what we suggest is an ideal cachexia animal model as described below.

Anorexia-cachexia syndrome in parathyroid hormone-related protein (PThrP)-secreting tumor bearing animals

PThrP is frequently produced and secreted by cancers and is a special type of leukemia/lymphoma (adult T cell leukemia/lymphoma). PThrP is known to be a principal factor responsible for humoral hypercalcemia
of malignancy (HHM) [49, 50].

Both polyclonal and monoclonal antibodies that neutralize PTHrP exerted anti-hypercalcemic and anti-cachectic effects in animal models [51–53]. Onuma et al. generated a humanized anti-PTHrP monoclonal antibody [54]. We demonstrated that peripheral administration of this antibody improved anorexia-cachexia syndrome in PTHrP-secreting tumor bearing animals [53–55]. In this rat model, the mRNA levels of orexigenic neuropeptides such as NPY were significantly higher than those found in nontumor-bearing rats, whereas mRNA levels of anorexigenic peptides such as POMC and CRH were lower than in nontumor-bearing rats [56]. Bolus intravenous administration of anti-PTHrP antibody returned these mRNA levels to control levels [56]. The results suggest that starvation caused these changes in the hypothalamus and improved feeding was a consequence of the restoration from the administration of anti-PTHrP antibody. In this model, the leptin level in plasma was markedly decreased and anti-PTHrP restored it.

Many appetite-regulating peptides are under the control of leptin [3, 35, 57–59]. Thus, it seems that adipose tissues and the hypothalamus are not the primary target sites of PTHrP to induce anorexia and cachexia. On the other hand, the mRNA levels of orexins and MCH that are expressed in the neurons of the LHA and enhance feeding [60, 61] were not markedly altered in this rat model and an anti-PTHrP antibody had little effect. PTHrP may affect factors and mechanisms that function between feeding-regulating neuropeptides in the hypothalamus and pathways in the central nervous system (CNS) downstream of these neuropeptides (Fig. 4). We cannot disregard the possibility that hypercalcemia in plasma may inhibit appetite and feeding through the CNS because severe hypercalcemia was observed in this model. However, there is a potent possibility that PTHrP may act as a neurotransmitter/neuromodulator in the CNS to inhibit feeding because PTHrP and its receptor, PTH1R mRNAs, were widely expressed in the brain of the rat [62].

Conclusions

The etiology of anorexia and appetite loss in cancer anorexia-cachexia syndrome is not explained by modification of the two centers theory in the hypothalamus and possible involvement of orexigenic and/or anorexigenic neuropeptides in the hypothalamus. Dehydration-induced anorexia and LPS-induced anorexia seem to be the result of the up-regulation of orexigenic neuropeptides in the hypothalamus and starvation signals that stimulate the up-regulation of orexigenic neuropeptides. However, it is very difficult to understand the etiology of feeding-regulating hypothalamic neuropeptides of cancer anorexia-cachexia syndrome. Unknown factors/pathways cause anorexia/cachexia, and anorexia-induced up-regulation of orexigenic neuropeptides and down-regulation of anorexigenic neuropeptides in the hypothalamus does not stimulate feeding. Why do hypothalamic neuropeptide signals that stimulate eating not cause feeding? Does a common chemical mediator such as PTHrP exist in anorexia-cachexia syndrome? A PTHrP-secreting tumor may give us new insight into cancer anorexia-cachexia syndrome. Further study is required to find a common factor responsible for cancer anorexia-cachexia syndrome and the action site of the anorexic signal in the CNS.

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References


