Involvement of Novel Feeding-Related Peptides in Neuroendocrine Response to Stress

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Various stressors are known to cause eating disorders. However, it is not known in detail about the neural network and molecular mechanism that are involved in the stress-induced changes of feeding behavior in the central nervous system. Many novel feeding-regulated peptides such as orexins/hypocretins and ghrelin have been discovered since the discovery of leptin derived from adipocytes as a product of the *ob* gene. These novel peptides were identified as endogenous ligands of orphan G protein–coupled receptors. The accumulating evidence reveals that these peptides may be involved in stress responses *via* the central nervous system, as well as feeding behavior. The possible involvement of novel feeding-related peptides in neuroendocrine responses to stress is reviewed here. Exp Biol Med 228:1168–1174, 2003

Key words: stress; peptides; G protein–coupled receptor; feeding

Experience has shown that stress is an inducing factor in transient loss of appetite or bulimia and chronic anorexia or overeating. Physiological reactions induced by stress include autonomic nervous responses such as elevated blood pressure, increase of heart rate, or decrease of gastrointestinal motion; endocrine responses such as activation of hypothalamopituitary adrenal (HPA) axis; emotional changes; and behavioral changes such as anorexia or bulimia.

Considerable studies have shown that classic neurotransmitters such as noradrenaline (NA), dopamine, and serotonin and stress hormones (corticotropin-releasing hormone [CRH]) have a contributory role. Recently, many peptides have been termed feeding-related peptides (Table I), and it is currently believed that these peptides, acting as feeding regulatory factors, contribute to various physiological stress reactions (Table II).

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G protein–coupled receptors for unknown endogenous ligands are called "orphan receptors." The method for finding endogenous ligand of an orphan receptor is called "reverse pharmacology" because its sequence is reversed from the conventional one in which a physiologically active substance is discovered and its receptor is then identified. Recently a series of novel endogenous ligands have been discovered using this method. Among the feeding-related peptides we discuss, orexins/hypocretins (1), galanin-like peptide (GALP) (2), prolactin-releasing peptide (PrRP) (3), and ghrelin (4) are peptides discovered by this method.

Feeding Regulation by Neuropeptides

Our primitive "appetite" is controlled by a feeding center and a satiety center in the hypothalamus (5). The classic neurotransmitters such as NA, dopamine, and serotonin as well as many neuropeptides play a role in the neural network at these sites. The medial part of the arcuate nucleus (Arc) of the hypothalamus includes numerous neurons that contain neuropeptide Y (NPY), a peptide that powerfully evokes feeding. NPY and agouti-related protein (AgRP) are both present in these neurons. The lateral part of the Arc includes neurons containing pro-opiomelanocortin (POMC) and functions as a feeding inhibition system. The lateral hypothalamic area, known as the feeding center, includes separate neurons containing orexins/hypocretins and melanin-concentrating hormone, respectively, and functions as a feeding stimulation system. The ventromedial nucleus of the hypothalamus is not known to include many neurons containing neuropeptides, but glucose-receptive neurons demonstrate leptin receptors. The hypothalamic paraventricular nucleus (PVN) comprises magnocellular and parvocellular neurons; the magnocellular neurons produce arginine vasopressin and oxytocin (OXT), project their axons into the posterior pituitary, and secrete them into the systemic circulation. The parvocellular neurons in the PVN produce peptides such as CRH and thyrotropin-releasing hormone (TRH) and project their axons into the median eminence, and the peptides they secrete into the hypophysial portal bloodstream control secretion of anterior pituitary hormones. The parvocellular neurons in the PVN also

Table I. Feeding Related Peptides

Anorexigenic peptides	Orexigenic peptides
Adrenomedullin	NPY
Bombesin	AgRP
CCK	Galanin
Calcitonin	GHRH
CART	GH
CRH	Ghrelin
Glucagon	MCH
GLP-1	Opioids (β-endorphin)
GRP	Orexin-A
Insulin	-B/hypocretin-1
Leptin	-2
Melanocortin	Prolactin
αMSH	Peptide YY
Neuromedin U	
Neurotensin	
Oxytocin	
PACAP	
POMC	
Somatostatin	
TRH	
Urocortin	
Urocortin II	
Urocortin III	
VIP	

include autonomic neurons with axons projecting to sympathetic preganglionic neurons of the intermediolateral spinal columns.

Feeding Regulation by Peripheral Peptides

After eating, digestive hormones such as cholecystokinin (CCK) transmit neural information regarding satiety to the hypothalamus. This information travels through the abdominal afferent vagus nerves distributed to the stomach and duodenum and is relayed through the nucleus of the tractus solitarius (NTS) and an intracranial neural circuit. Regulation of OXT secretion by CCK is a well-investigated response.

Leptin is produced by adipocytes and secreted into the systemic circulation. Leptin is believed to act on the satiety center in the hypothalamus to suppress feeding (6). Specifically, it functions as a feeding inhibition system by suppressing the neuronal activity of the Arc containing NPY and triggering accelerated activity of neurons containing POMC. It also promotes energy metabolism by stimulating the sympathetic nervous system.

Ghrelin is a peptide identified recently as an endogenous ligand of growth hormone secretagogue (GHS) analog receptors, and it is produced in large quantities by the stomach (7). Ghrelin in the systemic circulation not only brings about secretion of GH from the anterior pituitary, it also stimulates neuronal activity of the Arc containing NPY/AgRP and otherwise activates the feeding center in the hypothalamus.

Table II. Contribution of Peptides to Physiological Stress Reactions

	Response to stress	Effect on ACTH secretion
Orexigenic signals		
AgRP	?	Increase
Ghrelin	Activation	Increase
MCH	Activation	Increase
NPY	Activation	Increase
Orexin/hypocretin	Activation	Increase
Anorexigenic signals		
αMSH	Activation	Increase
CART	?	Increase
CCK	?	Increase
CRH	Activation	Increase
Leptin	Suppression or no change	Decrease or increase
Neuromedin U	?	Increase
Oxytocin	Activation	Decrease or increase
PrRP	Activation	Increase
TRH	?	Decrease?

Stress Responses and Feeding-Related Peptides

CRH and Urocortin. The center of the physiological endocrine response to stress is the HPA axis. CRH produced in the PVN triggers secretion of ACTH from the anterior pituitary, and ACTH released into the systemic circulation causes secretion of adrenocortical hormones (glucocorticoids) from the adrenal cortex. The fact that central administration of CRH causes various stress-induced stress responses in animal experiments indicates that CRH is a stress hormone in the central nervous system (CNS). Intracerebroventricular administration of CRH in rats also inhibits feeding behavior (8). There are two types of CRH receptors, termed type 1 receptors (CRHR-1) and type 2 receptors (CRHR-2). Endogenous ligands with high affinity for the CRHR-1 and CRHR-2 receptors have been investigated, and peptides identified as demonstrating high affinity to CRHR-2 receptors are urocortin (9), urocortin II (10), (stresscopin-related peptide) (11), and urocortin III (12) (stresscopin) (11). Urocortins II and III demonstrate more selective high affinity to CRHR-2 receptors than urocortin. Recent studies about CRH receptor knockout mice (13, 14) have shown that, in urocortin-induced feeding inhibition, the first half is mediated by CRHR-1 receptors, and the second half is mediated by CRHR-2 receptors. Urocortin II (stresscopin-related peptide) and urocortin III (stresscopin) have also been proven to have a feeding inhibition effect (10, 11).

Orexins/Hypocretins. Orexin-A and -B were discovered as endogenous ligands of G protein–coupled receptors, and hypocretin-1 and -2 were identified through a cDNA library search by a completely separate group. These were identical genetic products (1, 15). Since the discovery of orexins, a great deal of feeding-related research has been carried out on the basis of the localization of orexin-containing neurons in the lateral hypothalamic area and its surrounding area, the lateral hypothalamic area being known as the feeding center. Intracerebroventricular admin-

istration of orexins in the rat and mouse (1, 16) stimulates feeding, and fasting causes increase of orexin mRNA levels (1). It has been also reported that dietary intake is decreased in orexin gene knockout mice (17). However, the effect of intracerebroventricular administration of orexins on feeding is short term and does not change 24-hr total dietary intake or body weight (18, 19). The effect of orexins on feeding is currently attributed to evocation by orexin-induced stimulation of NPY-producing neuron activity in the Arc, where the axons of orexin-producing neurons project (20–25). Many orexin-producing neurons express leptin receptors, and leptin may also regulate the activity of orexin-producing neurons (26, 27).

The parvocellular neurons in the PVN producing CRH have demonstrated abundant expression of the orexin type-2 receptor gene, as well as projection of axons from orexin-producing neurons (28). Intracerebroventricular administration of orexins in the rat has produced the expression of the c-*fos* gene in the parvocellular neurons of the PVN (28–30), secretion of ACTH (29), and elevation of plasma concentration of corticosterone (29–32). The reaction of the secretions of ACTH and corticosterone is completely eliminated by preadministration of the CRH antagonist, α-helical CRH9-41 (32). It is also attenuated by preadministration of NPY antagonists and anti-NPY antibodies (7, 33). Intracerebroventricular administration of orexins in conscious rats increases face-washing behavior, grooming behavior, and exploratory behavior, and these behaviors are inhibited significantly by preadministration of α-helical CRH9-41 (31, 34). Northern blot analysis has demonstrated an increase in the orexin mRNA due to restraint stress and cold stress (31). These results continue to show that the central orexin system is related to physiological stress responses.

Neuromedin U. Neuromedin U (NMU), which is a 23-amino-acid neuropeptide, was discovered from the porcine spinal cord and other tissues (35–37). NMU is widely distributed in the peripheral organs and the CNS (38, 39). Peripheral administration of NMU causes elevation of blood pressure (35), alternation of ion transport (40), and regulation of the adrenocortical function (41). Intracerebroventricular administration of NMU suppresses food intake and heat production (42, 43).

Recent studies have demonstrated that NMU is an endogenous ligand of G protein–coupled receptors, NMU1R, and NMU2R (previously called as FM-3 and FM-4, respectively) (42, 44–45). NMU1R is expressed abundantly in the peripheral organs (42, 44–46), and the expression of NMU2R is mostly restricted to specific regions in the rat brain, in particular the PVN, the wall of the third ventricle in the hypothalamus, and the CA1 region of the hippocampus (42). Ozaki *et al.* demonstrated that intracerebroventricular administration of NMU caused activation of HPA axis and OXT release in rats (47). NMU may be involved in neuroendocrine responses to stress.

Galanin and Galanin-Like Peptide. Galanin is a 29-amino-acid peptide present in the hypothalamus in high concentrations (48). Intracerebroventricular administration or microinjection of galanin in rats evokes feeding and elevates fat feeding in particular (49). Galanin coexists with arginine vasopressin and CRH in the PVN. Microinjection of galanin in the rat PVN is reported to attenuate ACTH secretion elicited by stress (50). Galanin is known to coexist in the great number of NA-producing neurons in the locus ceruleus, and restraint stress is reported to increase the increase of the expression of the galanin gene (51). Galanin-containing neurons express glucocorticoid receptors, suggesting the relationship with the HPA axis.

GALP was recently identified among galanin receptor subtypes (GALR 1, 2, 3) as an endogenous ligand of GALR2 (2). A recent study demonstrated that dehydration and salt loading markedly increased in the expression of the GALP gene in the pituicytes of rat posterior pituitary (52). Recently, Saito *et al.* demonstrated that the expression of the GALP gene in the posterior pituitary is upregulated during nonosmotic stimuli such as endotoxin shock and chronic inflammatory stress (53).

Oxytocin. Intracerebroventricular administration of OXT inhibits feeding. Treatments intended to promote OXT secretion from the posterior pituitary (e.g., stress, peripheral administration of LiCl and CCK, and gastric distension) also inhibit feeding. CCK is regarded as the physiological satiety signal (54) and the pathway from CCK to OXT secretion is well investigated. First, CCK_A receptors in the stomach are stimulated, the abdominal vagus nerve is activated, NA neurons in the A2 region of the NTS are excited, and NA is released in the hypothalamus, which activates magnacellular OXT neurons (55–57). In addition to the A2 NA neurons (58), NA neurons in the medullary ventrolateral A1 region also play an important role in OXT secretion after stressful stimuli such as noxious stimuli (59). It is unlikely that OXT in the peripheral blood controls feeding directly. At the time when OXT secretion from the posterior pituitary is promoted, OXT release within the hypothalamus has also been shown to be increased, and OXT in the CNS has been shown to inhibit feeding (60). Intracerebroventricular administration of an OXT receptor antagonist attenuates feeding reduction in response to LiCl or CCK (61, 62) and blocks feeding reduction in response to CRH (63). These data suggest that intrinsic OXT may play an important physiological role in inhibition of feeding during satiety and stress. Studies with light microscopy have shown that nerve fibers containing the feeding-inhibition peptides, cocaine-amphetamine-regulated transcript (CART) (64) and PrRP (65), have synaptic contact with hypothalamic OXT neurons. OXT neurons are activated by CART or PrRP administration (66). It has also been suggested that α-melanocyte-stimulating hormone (α-MSH), a feeding inhibition factor released by POMC neurons, activates OXT neurons (67). It is therefore possible that OXT contributes to feeding inhibition by CART, PrRP, and α-MSH. The inhibitory effect of OXT on feeding is blocked by a GLP-1 receptor antagonist. OXT activates GLP-1 neurons in the brain stem. GLP-1

decreases food intake. It is thus possible that GLP-1 is a downstream mediator after activation of OXT neurons (68).

OXT has a short-term feeding inhibition effect. However, when OXT is administered for a long period, it has been reported that food intake becomes increased after an initial decrease in feeding (69). There are no reports that OXT knockout mice get fat. In addition, OXT receptor antagonists also do not block feeding inhibition by α_1NA receptor agonists (70).

The site of OXT action upon the feeding inhibition is not fully understood. Anatomically, OXT neurons in the PVN project to the medullary dorsal nucleus of the vagus nerve, and microinjection of OXT into the dorsal nucleus of the vagus nerve inhibits gastric motility, suggesting that OXT neurons of the PVN projecting to the medulla oblongata may act to inhibit feeding (60). OXT neurons in the PVN also project to sympathetic preganglionic neurons of intermediolateral nuclei in the spinal columns. OXT has been suggested to excite these neurons. Consequently, it is possible that activation of the sympathetic nervous system is a cause of feeding inhibition. OXT reduces binding affinity in the hypothalamus of α_2NA receptor agonists that have a feeding promotion effect (71). Thus, modification of NA receptors may also contribute to feeding inhibition by OXT. In addition to feeding inhibition, OXT released in the CNS after various stress stimuli has been proposed to modify neuroendocrine stress responses, such as ACTH secretion, and to affect anxiety behaviors (72).

Prolactin-Releasing Peptide. PrRP was identified as a factor causing release of prolactin from the anterior pituitary *in vitro* (3). PrRP-producing neurons are present in the NTS, the medullary ventrolateral areas, and the hypothalamic ventromedial and dorsomedial areas. The medullary PrRP-producing neurons are A2 or A1 NA neurons producing NA (73). Anatomic data suggest that PrRP fibers have synaptic contact with hypothalamic CRH neurons and OXT neurons (65). Intracerebroventricular administration of PrRP elicits ACTH release and OXT release from the anterior and posterior pituitary (66, 74). Stress activates medullary and hypothalamic PrRP neurons (75, 76). It has been suggested that PrRP and NA may both function cooperatively in neuroendocrine responses to stress (75). Intracerebroventricular administration of anti-PrRP antibodies to rats attenuates OXT secretion in response to conditioned fear (76).

The satiety factor, CCK, activates PrRP neurons. Intracerebroventricular or hypothalamic microinjection of PrRP inhibits feeding (77, 78) but does not induce nausea (conditioned taste aversion) (79). PrRP also increases body temperature. From these data, PrRP has been proposed to mediate satiety signaling (79). PrRP neurons express leptin receptors and PrRP mRNA is reduced when leptin signaling is defective (e.g., fasting, Zucker rats) (80). Thus PrRP is regulated by leptin. PrRP promotes release of the feeding inhibition factors, α-MSH and neurotensin (78). It is thus possible that α-MSH and neurotensin contribute to the inhibitory effect of PrRP. The action of PrRP on feeding inhibition during stress requires further investigation.

Leptin. Leptin synthesized by adipocytes acts on the brain to inhibit feeding (6). The targets of leptin in the brain are believed to be the Arc, the hypothalamic ventromedial nucleus, and the hypothalamic dorsomedial nucleus. Leptin receptors are present in these regions. For example, leptin receptors (OB-Rb) are present in NPY neurons of the Arc, which have a feeding promotion effect, and in POMC neurons of the Arc, which have a feeding inhibition effect. Leptin inhibits NPY neurons and activates POMC neurons. When leptin signaling is deficient, hypothalamic NPY and AgRP mRNA levels are increased, and POMC and CART mRNA levels are decreased. Leptin also raises energy consumption. This is partly due to the fact that leptin activates hypothalamic neurons projecting to sympathetic preganglionic neurons of intermediolateral nuclei in the spinal columns.

Leptin concentration in the blood decreases during hunger. In the state of hunger, the HPA system is activated, whereas thyroid, reproductive, and growth hormone systems are suppressed. Body temperature and energy metabolism decline. At such time, immune function is depressed. These responses are attributed to physiological adaptive phenomena responding to hunger (6). Decrease in leptin concentration may contribute to these responses. The HPA system is the primary system responding to stress, and the effect of leptin on the HPA system is deemed inhibitory. Intracerebroventricular administration of leptin attenuates stress-induced responses in hypothalamic CRH mRNA increase and ACTH release from the pituitary. Leptin has been suggested to inhibit activity of NPY neurons and NA release within the PVN (81), and NPY and NA have been shown to activate CRH neurons. Leptin may also reinforce glucocorticoid feedback inhibition by increasing the expression of PVN and hippocampal glucocorticoid receptors (82). It has also been suggested that leptin acts on adrenal cortex levels and inhibits glucocorticoid release (83, 84). On the contrary, some studies report that leptin activates the HPA axis (85, 86). Meanwhile, leptin production is stimulated by glucocorticoids, insulin, adrenocortical hormones, estrogen, and cytokines, whereas it is inhibited by adrenalin and testosterone.

Ghrelin. Ghrelin was discovered as an intrinsic ligand of GHS receptors (GHS-R) (4). Ghrelin is produced primarily in the stomach, but production in the colon, pituitary, kidneys, placenta, and hypothalamus has also been discovered. Ghrelin acts on the pituitary or the hypothalamus to bring about release of GHRH or an unknown factor (U-factor) and thereby promote GH secretion from the pituitary (87). Ghrelin also evokes a hunger sensation in the human and promotes feeding, perhaps *via* activation of GHS-R in a separate system. The blood concentration of ghrelin is decreased by satiation or feeding and is conversely increased during fasting or hunger or in anorexia nervosa. There is evidence that ghrelin acts on the hypothalamus to promote feeding. Microinjection of ghrelin to the Arc, the PVN, the

hypothalamic dorsomedial nucleus, and the hypothalamic ventromedial nucleus promotes feeding (88). Ghrelin neurons have been shown to lie in the space between the lateral hypothalamus, arcuate, ventromedial, dorsomedial, and paraventricular hypothalamic nuclei (89). Ghrelin administration activates NPY/AgRP neurons in the Arc (90), and the feeding promotion effect of ghrelin has been shown to be blocked by NPY Y1 receptor antagonists, antibodies to NPY, antibodies to AgRP, and antibodies to orexin (91); it is therefore possible that the feeding-promotive effect of ghrelin is mediated by NPY/AgRP neurons in the Arc and orexin neurons in the lateral hypothalamic area. However, the action of ghrelin has also been observed in NPY knockout mice. It also has been suggested that ghrelin stimulates the hypothalamic feeding center *via* activation of the vagus nerve (92).

Ghrelin receptors are distributed widely within the brain: the hypothalamus, hippocampus, substantia nigra, ventral tegmental area, and raphe nuclei. Ghrelin may therefore have physiological functions other than feeding. Ghrelin has the opposite effect of leptin in metabolism. Ghrelin decreases oxidation of fats and causes fat accumulation. The function of ghrelin during stress remains to be determined. A recent report states that ghrelin augments ACTH release in response to stress secretion-inhibiting effect of leptin (81). In this respect, it is interesting to point out that ghrelin reduces γ-aminobutyric acid release presynaptically in the PVN (89).

Conclusion

We summarize the topic of stress and feeding-related peptides with emphasis on recently discovered novel peptides. Future understanding of the contribution of feeding-related peptides to physiological stress responses holds the key to prevention and treatment of various stress-induced illnesses.

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