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Transgenic Approach to the Study of Body Weight Regulation

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***Abstract*——Energy homeostasis is accomplished through a highly integrated and redundant neurohumoral system. Recently, novel molecular mediators and regulatory pathways for feeding and body weight regulation have been identified in the brain and the periphery. Because of the multitude and complexity of disturbances in energy intake, expenditure, and partitioning that are associated with obesity, it has been difficult to determine which abnormalities are causative versus less important phenomena that are consequences of the altered neuroendocrine and metabolic milieu. Transgenic technology has provided new opportunities to modify the complex body weight-regulating system and to assess the relative importance of the individual components. Observations of mutant mice have shed new light on the understanding of energy homeostasis equation. Once created, transgenic animal models may be useful in assessing the efficacy or determining the mode of action of potential new therapeutic agents. However, the interpretation of targeted mutation is sometimes not straightforward in unraveling the physiology because of the redundancy and compensation of the regulatory machin-**

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ery, as well as the inherent problems of manipulation of the gene. Modifying the synthesis of a particular gene at all sites and developmental stages may be a relatively crude way of investigating its functions. Advanced gene-targeting strategies aimed at specific alterations (on and off) of a gene product at desired tissues and times could lead to a better understanding of the system.

I. Introduction

Most naturally existing single-gene mutations resulting in obesity in rodents have been cloned in recent years (Spiegelman and Flier, 1996; Chua and Leibel, 1997; Levine and Billington, 1998; York and Hansen, 1998). These mutations include yellow (Agouti, A^y), obese (*ob*/*ob*), diabetes (*db*/*db*), fat, tubby, and Zucker Fatty (*fa*/*fa*), which have been extensively studied in an effort to understand the physiological and biochemical basis for their obese phenotype. Obesity research has especially gathered momentum since the characterization of the obese (*ob*)[2] gene and its product leptin (Friedman and Halaas, 1998).

Energy homeostasis is accomplished through a highly integrated and redundant neurohumoral system that minimizes the impact of short-term fluctuations in energy balance on fat mass (Bray and York, 1979, 1998; Rohner-Jeanrenaud, 1995; Kalra, 1997; Elmquist et al., 1998,1999; Flier and Maratos-Flier, 1998; Friedman and Halaas, 1998; Woods et al., 1998; Kalra et al., 1999a; Inui, 1999a). Recently, novel molecular mediators and regulatory pathways for feeding and body weight regulation have been identified in the brain (Elmquist et al., 1998,1999; Flier and Maratos-Flier, 1998; Woods et al., 1998; Kalra et al., 1999a; Inui, 1999a). These have generated great interest in the genetic framework of body weight regulation and the derangement of the tight control of energy homeostasis leading to obesity or anorexia/cachexia.

[2] Abbreviations: ACTH, adrenocorticotropic hormone; AGRP, Agouti-related protein; α-MSH, α-melanocyte-stimulating hormone; aP2, adipocyte lipid-binding protein; ARC, arcuate nucleus of the hypothalamus; BAT, brown adipose tissue; BBB, blood-brain barrier; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; C/EBP, transcription factor CCAAT/enhancer-binding protein; CNS, central nervous system; CRF, corticotropin-releasing factor; DMH, dorsomedial nucleus of the hypothalamus; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GH, growth hormone; GHRH, growth hormone-releasing hormone; GLP-1, glucagon-like peptide-1_{7-36} amide; GLUT4, glucose transporter-4; GPDH, glycerol-3-phosphate dehydrogenase; GRP, gastrin-releasing peptide; 5-HT, 5-hydroxytryptamine serotonin; IL, interleukin; LH, lateral hypothalamus; MC, melanocortin; MCH, melanin-concentrating hormone; mRNA, messenger ribonucleic acid; NE, norepinephrine; POMC, proopiomelanocortin; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; ob, obese; PP, pancreatic polypeptide; PPAR, peroxisome proliferator-activated receptor; PTP, protein tyrosine phosphate; PVN, paraventricular nucleus of the hypothalamus; PYY, peptide YY; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; SNS, sympathetic nervous system; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor-α; TRH, thyrotropin-releasing hormone; UCP, uncoupling protein; VMH, ventromedial nucleus of the hypothalamus.

Transgenic technology, which permits the introduction of genes into the germ line of mice, and homologous recombinant gene knockout, which allows elimination of endogenous gene expression, are powerful tools for exploring the complex pathogenesis of obesity. The genetic manipulations have provided new models relevant to the study of each of the elements, as well as suggesting several illuminating, although sometimes confusing, insights into the underlying mechanisms (Levine and Billington, 1998; York and Hansen, 1998). The purpose of this review is to present recent advances in the understanding of body weight regulation, with a particular emphasis on transgenic animal models.

II. Leptin and Body Weight Regulation

The identification of the *ob* gene (Zhang et al., 1994) and the discovery that its encoded protein, leptin, is an adipocyte-derived hormone that is essential for normal regulation of body weight have greatly altered the field of metabolic physiology (Spiegelman and Flier, 1996; Flier, 1998). Leptin reduces appetite and increases energy expenditure when injected peripherally or i.c.v., and evidently elicits these effects via the central nervous system (CNS; Elmquist et al., 1998, 1999; Flier and Maratos-Flier, 1998; Friedman and Halaas, 1998; Sawchenko, 1998; Inui, 1999a). Leptin enters the brain by an active saturable system (Halaas et al., 1995; Banks et al., 1996) and acts through or in concert with several neuropeptides, monoamines, and other transmitter substances that affect food intake in the brain-gut axis (Figs. 1 and 2).

Leptin concords well with the postulate of a lipostatic system for weight control, which was proposed to explain the relative stability of weight over time in many animal species as well as their capacity to respond well to short-term fluctuations in energy balance to restore body weight to previous levels. Leptin is an afferent signal from the periphery to the brain in a homeostatic feedback loop that regulates adipose tissue mass (Figs. 1 and 2; Schwartz et al., 1992; Bray and York, 1998; Flier, 1998; Friedman and Halaas, 1998). 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. Rising levels of leptin signal the brain that excess energy is being stored, and this signal brings about adaptations of decreased appetite and increased energy expenditure that resist obesity. Transgenic overexpression of leptin in the liver by using the human serum amyloid *P* component promoter has resulted in markedly de-

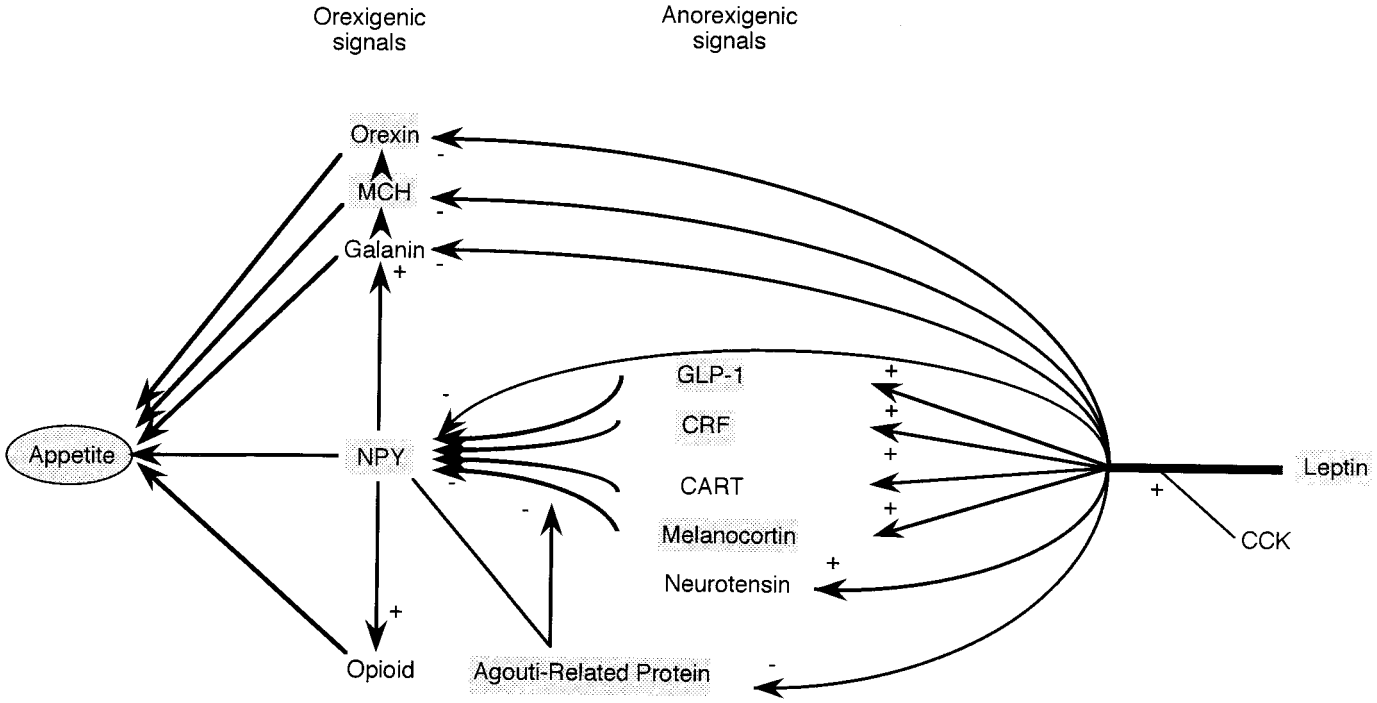

FIG. 1. A simplified cascade model for the interaction of leptin with hypothalamic neuropeptidergic effector molecules. Leptin acts as part of a feedback loop to maintain constant stores of fat (see also Fig. 2). A loss of body fat leads to a decrease in leptin, which activates feeding-stimulatory molecules in the hypothalamus, such as NPY. Conversely, an increase in body fat leads to an increase in leptin, which activates feeding-inhibitory molecules such as MC. NPY is the key component of the interconnected orexigenic network and its secretion and action are regulated by anorexigenic neuropeptides. In gray are the molecules of which the impact on body weight have been shown with either acute administration studies or chronic transgenic studies. MCH and orexin may situate downstream of NPY signaling. Other suggested interplays include those between leptin→TRH, TRH→NPY, GLP-1→CRF, α-MSH→MCH, and galanin→opioid, and also those in the opposite directions to the ones described in the figure, such as NPY→CRF and NPY→MC. These might have implications for the regulation of feeding and body weight, as well as for the activation of the hypothalamic-pituitary-adrenal axis. +, stimulatory input; −, inhibitory input

creased food intake and body weight gain with the complete disappearance of white adipose tissue and brown adipose tissue (BAT; Ogawa et al., 1999). Conversely, a loss of body fat leads to a decrease in leptin, and the physiological response is to increase appetite and decrease energy expenditure, both of which induce a positive energy balance and weight gain. *Ob*/*ob* mice, homozygous for a spontaneous mutation on the *ob* gene, failed to produce leptin and exhibited hyperphagia and obesity. Mutations in leptin receptors seen in *db*/*db* mice and *fa*/*fa* rats, resulted in an obese phenotype identical with that of *ob*/*ob* mice (Friedman and Halaas, 1998).

Shortly after the discovery of leptin it was also found that, with the exception of the *ob*/*ob* mice, obese rodents exhibit increased levels of serum leptin (Maffei et al., 1995; Frederich et al., 1995). The concept that obese rodents and humans are resistant to their endogenous leptin began to emerge. Recent studies have suggested that leptin resistance, also referred to as reduced leptin sensitivity, may play a significant role in the development of obesity (Strader et al., 1998). It may result not only from a structural aberration in the leptin receptor, but also from defective transport of leptin across the blood-brain barrier (BBB) and/or defects localized downstream in the signal transduction pathway of leptin (Caro et al., 1996; Bjorbaek et al., 1998). The observation of central leptin responsiveness in obese rodents in the face of peripheral leptin resistance may suggest a role for the reduced efficacy of leptin transport to the CNS (Van Heek et al., 1997).

III. Neuropeptidergic Cascade Downstream of Leptin Signaling

There is now a growing recognition that expression of appetite is chemically coded in the hypothalamus (Bray and York, 1979, 1998; Rohner-Jeanrenaud, 1995; Elmquist et al., 1998, 1999; Sawchenko, 1998; Woods et al., 1998; Inui, 1999a; Kalra et al., 1999a). Classic studies described syndromes of ravenous overeating and obesity as a consequence of lesions centered in the ventromedial nucleus (VMH; Hetherington and Ranson, 1940) and of a failure to eat and drink after damage to the lateral hypothalamus (LH; Anand and Brobeck, 1951), the dual-center model (Stellar, 1954). It is now known that other hypothalamic sites such as the paraventricular nucleus (PVN) and dorsomedial nucelus (DMH) also contain neural mechanisms that affect feeding behavior (Hetherington and Ranson, 1940; Stellar, 1954; Gold, 1973; Swanson and Sawchenko, 1983; Bernardis and Bellinger, 1996; Fig. 2). There are terminal fields of

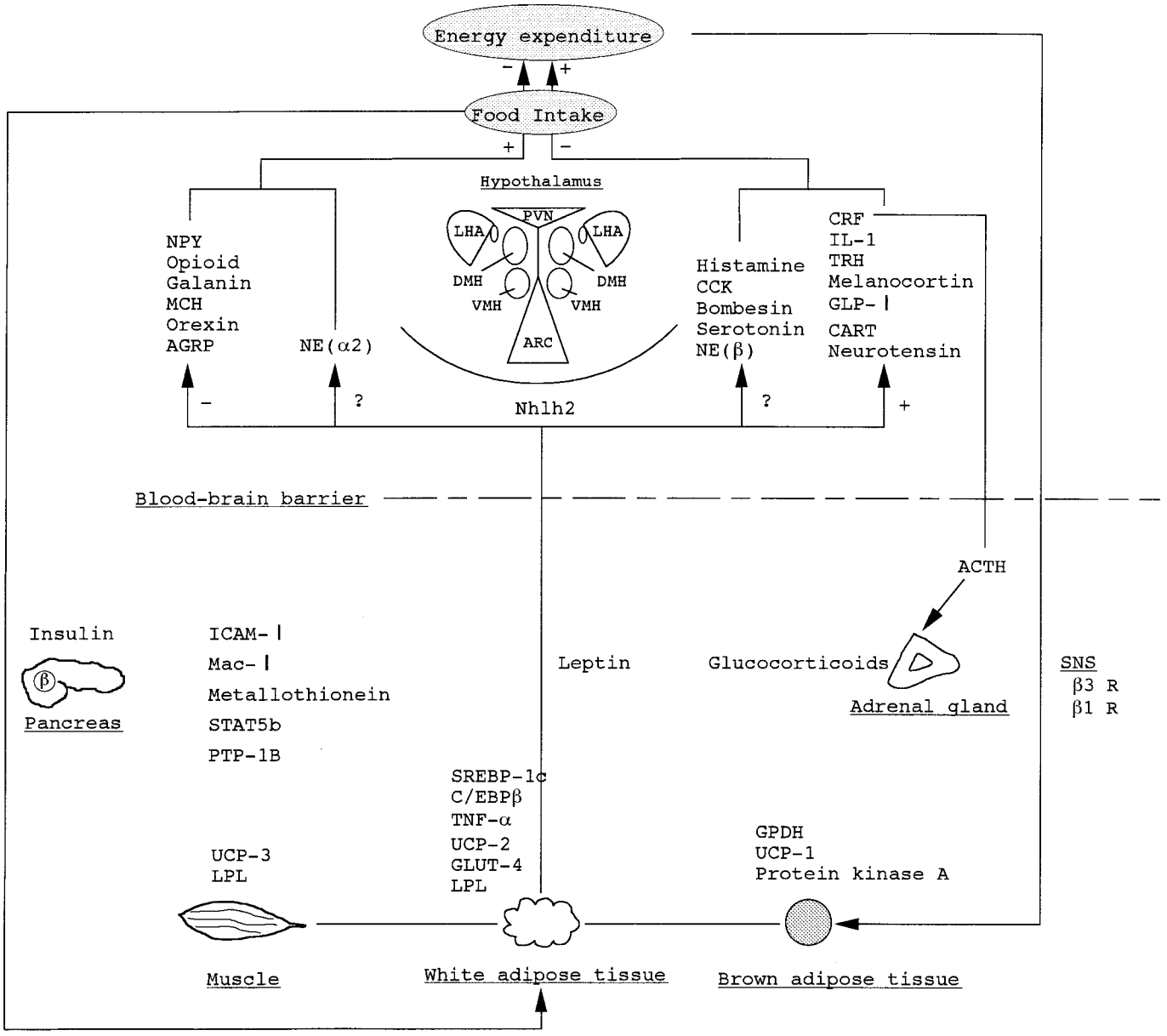

FIG. 2. Candidate molecules that control energy intake, expenditure and/or partitioning. The feeding-inhibitory molecules stimulate energy expenditure via the SNS-UCP axis, whereas the opposite applies to the feeding-stimulatory molecules. An increase in sympathetic activity is associated with an increase in the levels of UCP-1 mRNA in BAT, which produces thermogenesis, leading to reduced storage of fat. Genetic manipulation identified previously unknown regulators of energy homeostasis, including intracellular adhesion molecule-1, leukocyte integrin $\alpha M\beta 2$, metallothionein, and transcription factor Nhlh2, although fuller characterizations of these obese phenotypes need to be performed. β_1 R, β_1 adrenergic receptor; β_3 R, β_3-adrenergic receptor; LPL, lipoprotein lipase. Recently hypothalamic histamine was demonstrated to be involved in leptin signaling pathway (Yoshimatsu et al., 1999).

neurons from the arcuate nucleus (ARC), which is located at the base of the hypothalamus and contains orexigenic (feeding-stimulatory) and anorexigenic (feeding-inhibitory) neurotransmitters and neuromodulators. The biologically active, long form of the leptin receptor is produced in various hypothalamic sites including ARC, VMH, DMH, PVN, and LH (Mercer et al., 1996; Schwartz et al., 1996; Friedman and Halaas, 1998).

Figure 1 shows a simplified model for the interaction of leptin with hypothalamic neuropeptidergic effector molecules within a regulatory feedback loop. The model is based on the findings obtained mostly from acute administration studies, and it emphasizes the feeding drive systems that would underlie both obesity and hypothalamic response to starvation. Neuropeptide Y (NPY) is the most potent orexigenic peptide activated by the fall of leptin, and consists of an interconnected orexigenic network that includes galanin, opioid peptides, melanin-concentrating hormone (MCH), orexin, and agouti-related protein (AGRP; Morley, 1987; Inui et al., 1991; Qu et al., 1996; Flier and Maratos-Flier, 1998; Sakurai et al., 1998; Woods et al., 1998; Inui, 1999a,b; Kalra et al., 1999a). Most of these peptides are upregulated in *ob*/*ob* mice, and their expressions are in-

creased through fasting in wild-type mice and are inhibited by leptin administration (Inui, 1999a). Other effector molecules functioning in this homeostatic loop are the anorexigenic neuropeptides such as corticotropin-releasing factor (CRF), melanocortin (MC), glucagon-like peptide-$1_{7–36}$ amide (GLP-1), neurotensin, and cocaine- and amphetamine-regulated transcript (CART), the expression of which is down-regulated in *ob/ob* mice and stimulated by leptin (Schwartz et al., 1995; Flier and Maratos-Flier, 1998; Woods et al., 1998; Inui, 1999a; Kalra et al., 1999a). The administration of the receptor antagonists of these peptides effectively blocks the reduction of food intake and body weight induced by leptin (Inui, 1999a). An imbalance in the operation of either orexigenic or anorexigenic pathways is thought to perturb the regulatory microenvironment, leading to hyperphagia and abnormal weight gain (Kalra et al., 1999a). It has yet to be determined by which mechanisms the orexigenic network escapes the inhibitory influences of leptin and anorexigenic signals.

The orexigenic and anorexigenic substances decrease and increase sympathetic nervous activity, respectively, thereby regulating energy expenditure and body fat stores (Fig. 2). This is achieved by modulating thermogenesis in BAT and possibly in other sites such as white adipose tissue and muscle, through induction of the mitochondrial uncoupling protein UCP-1 and the newly identified UCP-2 and UCP-3 (Rohner-Jeanrenaud, 1995; Collins et al., 1996; Spiegelman and Flier, 1996; Fleury et al., 1997; Gong et al., 1997; Bray and York, 1998). The reciprocal relationship between food intake and sympathetic activity has been shown to be robust among various neurotransmitter substances (Bray, 1993; Bray and York, 1998). Other neurotransmitters that affect food intake and energy expenditure include feeding-stimulatory norepinephrine (via α_2-receptor) and γ-aminobutyric acid (GABA), and feeding-inhibitory serotonin and dopamine (Table 1). Neuropeptides are important components of the feeding-regulatory systems.

TABLE 1
Hormones, neurotransmitters, and neuropeptides that affect food intake
The feeding-stimulatory molecules include norepinephrine, γ-aminobutyric acid, and seven classes of neuropeptides, whereas the feeding-inhibitory molecules include serotonin, dopamine, and a long list of brain-gut peptides.

Stimulatory
Norepinephrine (NE, α_2 receptor)
γ-Aminobutyric acid (GABA)
Neuropeptide Y (NPY)
Opioids
Galanin
Growth hormone-releasing hormone (GHRH)
Melanin-concentrating hormone (MCH)
Orexin
Agouti-related protein
Inhibitory
Norepinephrine (NE, β receptor)
Serotonin (5-HT)
Dopamine
Histamine
Corticotropin-releasing factor (CRF)
α-Melanocyte-stimulating hormone (α-MSH)
Glucagon-like peptide $1_{7–36}$ amide (GLP-1)
Cocaine- and amphetamine-regulated transcript (CART)
Neurotensin
Cholecystokinin (CCK)
Bombesin
Carcitonin-gene related peptide (CGRP)
Amylin
Pancreatic polypeptide (PP)
Adrenomedullin
Glucagon
Enterostatin
Oxytocin
Anorectin
Thyrotropin-releasing hormone (TRH)
Cyclo-histidyl proline diketopiperazine (Cyclo-His-Pro)
Pituitary adenylate-cyclase activating polypeptide (PACAP)
Acidic fibroblast growth factor
Interleukin 1β (IL-1β)

IV. Key Components in Body Weight Regulation and Implications of Transgenic Animal Models

A. Hypothalamic Stimulators of Food Intake

1. NPY. NPY, a 36-amino acid peptide, is one of the most abundant and widely distributed neurotransmitters in the mammalian brain (Tatemoto, 1982; Sahu and Kalra, 1993; Billington et al., 1994; Leibowitz, 1995; Kalra, 1997; King and Williams, 1998; Inui, 1999b; Kalra et al., 1999a; Table 2). The ARC is the major site of expression for NPY within neurons in the hypothalamus that project to PVN, DMH, LH, and other hypothalamic sites. Although NPY can produce diverse effects on behavior and other functions, its most noticeable effect is the stimulation of feeding after central administration (Sahu and Kalra, 1993; Billington et al., 1994; Kalra, 1997; King and Williams, 1998; Inui, 1999b; Kalra et al., 1999a). The feeding-stimulatory effect of NPY is approximately 500 times more potent on a molar basis than norepinephrine (King and Williams, 1998). Multiple injections of NPY into the PVN or cerebral ventricle result in obesity, indicating that NPY is capable of overriding powerful inhibitory signals on food intake and body adiposity (Stanley et al., 1986; Stanley, 1993). NPY produces a shift to positive energy balance by increasing food intake, by decreasing energy expenditure primarily with a reduction in thermogenesis in BAT (Egawa et al., 1991), and by facilitating fat deposition in white adipose tissue partly through increased insulin activity (Zarjevski et al., 1993).

NPY synthesis in the ARC and its release into the PVN, the most abundant projection, are regulated by afferent signals such as leptin, insulin (both inhibitory), and glucocorticoids (stimulatory; Schwartz et al., 1992; Sahu and Kalra, 1993; Stanley, 1993; Leibowitz, 1995; Kalra, 1997; King and Williams, 1998; Inui, 1999b; Kalra et al., 1999a). NPY synthesis and secretion are all up-regulated in models of energy deficiency or increased

TABLE 2
Hypothalamic stimulators of food intake and their effect on body weight regulation

	Amino Acid Length	Site of Production	Site of Action	Effect on Body Weight			
				Administration	Antagonist	Overexpression	Knockout
NPY	36	ARC	PVN, PFH	↑	↓	→	→
β-endorphin	31	ARC	PVN, VMH[a]	→	↓	?	↑
Galanin	29–30	PVN	PVN	→	→	?	→
MCH	19	LH	PVN, LH[b]	→	?	?	↓
Orexin A and B	33 and 28	LH	LH, PVN, PFH	↑	?	↓	↓
Agouti-related protein	50	ARC	PVN	↑	↓	↑	?
Norepinephrine (α_2-receptor)	—	Brainstem	PVN	↑	↓	?	→ (Dopamine β-hydroxylase)

PFH, perifornical hypothalamus; ↑, increase; ↓, decrease; →, no change; ?, not reported.
[a] Response to injection is delayed (Baile et al., 1986).
[b] Decrease in food intake is reported (Presse et al., 1996).

metabolic demand such as starvation, insulin-dependent diabetes mellitus, lactation and physical exercise (Sahu and Kalra, 1993; Stanley, 1993; Kalra, 1997; Turton et al., 1997; Inui, 1999b; Kalra et al., 1999a). The NPY neurons that are activated by fasting are the neurons that express the long form of the leptin receptor (Baskin et al., 1999). A primary physiological role of the ARC NPY neurons may thus be to restore normal energy balance and body fat stores under conditions of energy deficit, the signals of which are falling leptin and/or insulin occurring in these conditions.

It is also known that hyperphagia and obesity in several genetic and experimental models are associated with augmentation of NPYergic signaling. Genetically obese *ob*/*ob* mice, *db*/*db* mice, and *fa*/*fa* rats exhibit increased preproNPY mRNA in the ARC and increased NPY levels and release in the PVN (Sanacora et al., 1990; Wilding et al., 1993; Dryden et al., 1995). Augmented NPY gene expression in the ARC and increased NPY levels as well as release in the PVN are observed in streptozotocin-induced diabetic rats, which may precede the onset of hyperphagia (Williams et al., 1988, 1989; Sahu et al., 1990, 1992, 1997). Arcuate NPY mRNA expression may also be abnormally high and not responsive to energy restriction before phenotypic expression of obesity in diet-induced obese rats (Levin and Dunn-Meynell, 1997; Levin, 1999). In this model, once obesity is fully expressed, NPY levels are depressed but become responsive to energy restriction to defend the higher body weight. Lesions in the VMH are associated with hyperphagia and body weight gain in rodents and humans (Hetherington and Ranson, 1940; Bray and York, 1979). However, preproNPY mRNA levels in the ARC and NPY levels as well as release from the PVN were unexpectedly suppressed in hyperphagic VMH-lesioned rats (Kalra, 1997; Dube et al., 1999). Obesities induced by neurotoxins such as colchicine, gold thioglucose, and monosodium glutamate also exhibit suppressed hypothalamic NPY gene expression and NPY levels or release in the PVN (Bergen and Mobbs, 1996; Stricker-Krongrad et al., 1996; Jain et al., 1998). However, feeding in response to NPY was increased and hyperphagia was suppressed by blockade of NPY or NPY receptors (Dube et al., 1995; Bergen and Mobbs, 1996; Stricker-Krongrad et al., 1996; Kalra et al., 1997, 1998). Both low and high abundance of NPY can be associated with hyperphagia and obesity (Kalra et al., 1999a). Increased NPY receptor abundance and sensitivity, and up-regulation of NPY in novel hypothalamic sites such as DMH may underlie the hyperphagia (Kalra et al., 1997, 1998, 1999a). Furthermore, increased induction and responsiveness of other orexigenic signals such as galanin were reported in colchicine-treated rats (Kalra et al., 1999a; Pu et al., 1999a). Therefore, diminution of leptin feedback or leptin resistance contributes to the hyperphagia and obesity through modifications of the NPY orexigenic network.

In view of the above evidence indicating a key role for NPY in energy homeostasis, it was surprising that mice in which the NPY gene had been deleted by homologous recombination were phenotypically normal except for an increase in susceptibility to seizures (Erickson et al., 1996a; Palmiter et al., 1998). These NPY-deficient mice were sensitive to the anorectic effect of leptin, indicating that leptin acts through pathways other than those involving NPY. Subsequent experiments with mice with a deficiency in both leptin and NPY demonstrated that the double mutant mice are halfway between normal lean and *ob*/*ob* mice in terms of body weight and fat mass, indicating that NPY is required for full expression of the ob phenotype (Erickson et al., 1996b). The absence of NPY, however, did not attenuate the development of obesity induced by a high-fat diet, chemical lesions of the hypothalamus, impaired BAT function due to a diphtheria toxin transgene or the lethal agouti mutation (A^y; Hollopeter et al., 1998a). The responses of NPY-deficient mice to anorectic and orexigenic substances including NPY, CRF, dexfenfluramine (an enhancer of serotonergic transmission), and MT (a melanocortin-4 receptor agonist) were unaltered although the initial response to galanin might be lost (Hollopeter et al., 1998b). It remains to be determined whether NPY is involved in hyperphagia and obesity only under extreme conditions such as in *ob*/*ob* mice (Hollopeter et al., 1998a) or whether the normal phenotype is due to compensation by other orexigenic signals that replace NPY and maintain seemingly normal feeding and body weight regulation.

NPY transgenic animals were also created by using a novel CNS neuron-specific expression vector of human Thy-1 gene fragment linked to mouse NPY cDNA (Inui et al., 1998) or additional copies of the NPY structural gene introduced into the mouse (Thiele et al., 1998) or the rat (Michalkiewicz et al., 1999). NPY-overexpressing mice showed anxiety-like behavior via CRF neuronal system (Inui et al., 1998) or decreased ethanol consumption with increased sensitivity to ethanol-induced sedation (Thiele et al., 1998). Ethanol consumption and resistance appeared to be inversely related to NPY levels (Thiele et al., 1998). However, they exhibited seemingly normal food intake and body weight regulation, although the transgene expression may be minimal in the hypothalamus of these animals. Transgenic rats also showed only gender-dependent increases or decreases in food intake and body weight despite the hypertension and enhanced cardiovascular responsiveness to adrenergic stimulation and stress (Michalkiewicz et al., 1999).

NPY activates at least six G protein-coupled receptor subtypes, Y_1 to Y_6, all of which have been cloned except for the Y_3 receptor (Balasubramaniam, 1997; Blomqvist and Herzog, 1997; Gehlert, 1998; Michel et al., 1998). NPY analogs exhibit varying degrees of affinity and specificity for these Y receptors, as well as potency in stimulating feeding (Inui, 1999b). Recent studies on NPY have focused on the Y receptors, because new antiobesity drugs may emerge from pharmacological characterization of the Y receptors and their antagonists. The Y_5 receptor has been isolated as the receptor that has pharmacological properties most closely matching a proposed feeding receptor (Gerald et al., 1996; Schaffhauser et al., 1997; Criscione et al., 1998). However, recent progress in the development of nonpeptide ligands suggests that NPY receptors other than the Y_5 may mediate the appetite-stimulating effect of NPY. The potent anorectic effects of Y_1 receptor-specific antagonists in various rodent models of obesity support the initial suggestion that the Y_1 receptor is involved in appetite regulation (Stanley, 1993; Kanatani et al., 1996, 1998; Lopez-Valpuesta et al., 1996; Ishihara et al., 1998; Wieland et al., 1998). It is also postulated that another Y receptor closely related to Y_1 and Y_5 may have a role in mediating feeding induced by NPY (Inui, 1999b; Kalra et al., 1999a).

Recently, gene knockout experiments were performed on the Y_1 and Y_5 receptors (Marsh et al., 1998; Pedrazzani et al., 1998; Kanatani et al., 1999). Both types of mutant mice fed and grew normally, although they developed unexpected late-onset obesity due to hyperphagia in Y_5-deficient mice or lowered metabolic rate associated with reduced locomotor activity in Y_1-deficient mice. However, the Y_1 receptor-deficient mice exhibited a markedly reduced feeding response to fasting, as well as a slightly reduced daily food intake and NPY-stimulated feeding. The Y_5 agonist (Peptide YY$(PYY)_{3–36}$)-induced feeding response, as well as fasting-induced corticosterone response (Pralong et al., 1999) were reduced in this mouse model. Y_1-receptor deficiency also led to impaired insulin secretion to glucose and changes in UCP expression such as up-regulation of UCP-1 in BAT and down-regulation of UCP-2 in white adipose tissue (Kushi et al., 1998). The Y_5 receptor deficient mice exhibited normal responses to fasting and leptin, but the feeding response to $PYY_{3–36}$ was markedly reduced and the residual response was eliminated by the simultaneous administration of the Y_1 receptor antagonist. Although shifts in the orexigenic network may be involved in the late-onset obesity in Y_1 and Y_5 receptor mutant mice, these observations indicate that both Y_1 and Y_5 receptors are involved in the regulation of feeding, but Y_1 receptor may play a more prominent role in mediating feeding induced by NPY. The Y_5 receptor appears not to be required for the development of the *ob*/*ob* obesity syndrome, because mice deficient for both leptin and the Y_5 receptor were indistinguishable from littermate *ob*/*ob* mice in body tempeature, body weight, food intake, and adiposity (Marsh et al., 1998). The Y_2 receptor is a predominant form of NPY receptors in the brain that is thought to be presynaptic and to suppress release of transmitters such as norepinephrine and NPY in the hypothalamus (Broberger et al., 1997; Blomqvist and Herzog, 1997; Michel et al., 1998; Gehlert, 1998). The Y_2 receptor subtype may have either a minor or no role in the regulation of feeding (Stanley, 1993). The Y_2 receptor agonist has no effect on feeding after i.c.v. administration, although it might reduce nocturnal food intake, especially the carbohydrate component of the meal, after injection into the PVN (Leibowitz and Alexander, 1991; Inui, 1999b). Very recently, the Y_2 receptor subtype has been inactivated. The mutant mice developed mild obesity caused by hyperphagia and displayed an attenuated feeding response to leptin but a normal response to fasting (Patrik, 1999). The observations indicate the importance of the Y_2 receptor in feeding and body weight regulation and show that it is an essential mediator of the leptin response. The Y_2 receptor was also found to be important in the basal control of heart rate (Patrik, 1999). The identification of NPY feeding receptors and their complimentary and/or overlapping functions is obviously of great importance to the molecular basis of the hypothalamic regulation of feeding and body weight. The gene knockout experiment on the respective Y receptors singly or in combination could be particularly useful for addressing this.

2. MCH. A population of neurons in the LH and zona incerta produce a cyclic 19-amino acid peptide, MCH, which was initially discovered in salmon pituitaries as a regulator of skin color change (Vaughan et al., 1989; Nahon, 1994). MCH potentiated nocturnal feeding after central administration, and MCH gene expression was stimulated by fasting and augmented in *ob*/*ob* mice (Qu et al., 1996; Rossi et al., 1997). However, MCH-induced feeding was small and of short duration relative to NPY,

and chronic administration had no effect on daily (cumulative) food intake and body weight (Rossi et al., 1997). It was even reported that MCH had a potent anorectic effect after administration into the cerebral ventricle or the zona incerta-LH area, which was highly dependent on the light/dark cycle (Presse et al., 1996). However, ablation of the gene led to a thin phenotype associated with reduced food intake and an inappropriately increased metabolic rate, indicating a role of MCH on the energy homeostasis equation (Shimada et al., 1998). MCH-deficient mice showed reduced amounts of leptin and pro-opiomelanocortin (POMC) mRNA in the ARC. Because deletion of a single gene encoding an orexigenic peptide can result in leanness despite the interconnected orexigenic network, MCH may act downstream of leptin and NPY signaling cascade, as might be expected from the immunohistochemical demonstration of projection from NPY neurons in the ARC to MCH neurons in the LH (Broberger et al., 1998a; Elias et al., 1998).

3. Orexin. The orexins are a recently identified class of neuropeptides that also were described as hypocretins (Sakurai et al., 1998; De Lecea et al., 1998). Orexin A and orexin B are 33- and 28- amino acid peptides, respectively, sharing 46% identity. Both peptides are coded by the same gene, and are localized in neurons in the dorsal and lateral hypothalamic areas and perifornical hypothalamus. Administration i.c.v. of orexin A and orexin B stimulated feeding in a dose-related fashion, with orexin A significantly more effective than orexin B, possibly through activation of both orexin A and orexin B receptors (Sakurai et al., 1998). Microinjection studies indicated that orexins act in the limited areas in the hypothalamus such as the LH, perifornical hypothalamus, and PVN, despite broad distribution of orexin fibers in hypothalamic and extrahypothalamic sites (Edwards et al., 1999; Kalra et al., 1999a; Sweet et al., 1999). However, orexin was found to be less effective than NPY in stimulating feeding (Edwards et al., 1999; Kalra et al., 1999a). Orexin may be more likely involved in the control of energy metabolism than of food intake (Lubkin and Stricker-Krongrad, 1998). Although fasting up-regulated orexin gene expression in the hypothalamus, down-regulation of the gene expression was observed in the *ob*/*ob* and *db*/*db* mice (Yamamoto et al., 1999). However, this may be due, in part, to the accompanying hyperglycemia in the animals because leptin acutely inhibits orexin gene expression. Very recently, orexin-overexpressing or -deficient mice were created, and both types of the mutant mice showed decreased body weight (T. Sakurai and M. Yanagisawa, personal communication). Orexin-overexpressing mice have reduced body weight despite increased food intake due to an inappropriately increased metabolic rate, and orexin-deficient mice have slightly reduced body weight despite markedly reduced food intake due to a decreased metabolic rate. The gene knockout experiment, together with established synaptic contacts between NPY neurons in the ARC and orexin neurons in the LH, suggests that orexin may also function as a downstream effector molecule of NPY signaling (Broberger et al., 1998a; Elias et al., 1998; Horvath et al., 1999).

4. Galanin. Galanin is a 29-amino acid peptide that is distributed in discrete subpopulations in the ARC, DMH, and PVN of the hypothalamus (Leibowitz, 1989, 1995). Galanin stimulates feeding in rats after injection into the cerebral ventricle, as well as into the PVN, LH, VMH, and central nucleus of the amygdala (Kyrkouli et al., 1990a; Schick et al., 1993; Corwin et al., 1993). Like MCH and orexin, galanin-induced feeding is less remarkable than that of NPY, and continuous galanin infusion was ineffective in inducing sustained hyperphagia and obesity (Smith et al., 1994). A close anatomical and functional relationship exists between neurons producing galanin and other orexigenic signals (Horvath et al., 1996). NPY neurons are in direct contact with galanin neurons in the ARC and PVN, and galanin may partly mediate NPY-induced feeding (Kalra et al., 1999a). Involvement of β-endorphin and norepinephrine (NE) in galanin-induced feeding was also suggested immunohistochemically, as well as from the attenuated feeding response to galanin by pretreatment with naloxone, an opioid receptor antagonist and rauwolscine, an α_2-adrenergic receptor antagonist, respectively (Kyrkouli et al., 1990b; Dube et al., 1994). Although the NPY system is closely associated with carbohydrate ingestion and use, channeling nutrients toward the synthesis of fat, galanin may function primarily in controlling fat ingestion and enhancing fat deposition through a reduction in energy expenditure (Leibowitz, 1995). Galanin may be active during the middle period of the natural feeding cycle, and a high-fat diet can enhance galanin production in the PVN, which was closely linked to body adiposity (Akabayashi et al., 1994; Leibowitz et al., 1998). Galanin may also be involved in hyperphagia seen in VMH regioned animals (Pu et al., 1999a; Kalra et al., 1999b). However, it needs to be further clarified how galanin constitutes an important orexigenic signal in natural feeding as well as hyperphagia in genetically obese rodents (Beck et al., 1993; Corwin et al., 1995). It was recently reported that galanin-deficient mice have markedly reduced synthesis and secretion of prolactin in the hypothalamus but they grow normally and have unaltered NPY and GLP-1 content in the hypothalamus (Wynick et al., 1998).

5. Opioid Peptides. The opioid system is composed of three families of biologically active peptides, β-endorphin, dynorphin, and enkephalins, and their receptors, μ-opioid receptor, κ-opioid receptor, and δ-opioid receptor, respectively (Levine and Billington, 1989, 1997; Mansour et al., 1995; Kalra et al., 1999a; Kieffer, 1999). Novel μ-selective endomorphins have also been identified in the brain (Zadina et al., 1997). One of the many functions of opioid peptides in the brain is involvement

in mediation of the hunger component in the control of food intake (Baile et al., 1986). Opioid peptides may potentiate fat as well as protein ingestion (Leibowitz, 1992). β-Endorphin, derived from precursor POMC, and dynorphin from prodynorphin, stimulate feeding after central administration (Baile et al., 1986; Morley, 1987; Inui et al., 1991; Lambert et al., 1993a; Kalra, 1997; Kalra et al., 1999a). POMC neurons are localized in the ARC and innervate the PVN, VMH, and other areas of the hypothalamus, where microinjection of β-endorphin and opiate agonists that bind to the μ-opioid receptors stimulate feeding (Baile et al., 1986; Kalra et al., 1999a). Dynorphin-producing neurons are also found in various regions of the hypothalamus, including the ARC and PVN. The opioid receptor antagonists, especially the μ- and κ-antagonists, decreased feeding in animals and humans (Morley, 1987; Cole et al., 1995). Antagonists such as naloxone and naltrexone decreased body weight during chronic administration, and were more potent in decreasing food intake and weight gain in obese than in lean rodents (Baile et al., 1986). β-Endorphin reduced sympathetic nerve activity in BAT, suggesting a potential role for opioids in thermogenesis (Egawa et al., 1993). Although the opioid-evoked feeding is modest, β-endorphin in particular may represent an important interconnected orexigenic signal (Kalra et al., 1999a). β-endorphin may situate downstream from NPY, galanin, and GABA because all three molecules stimulate β-endorphin release in the hypothalamus, and opioid antagonists such as naloxone inhibit feeding stimulated by any one of the three (Morley, 1987; Lambert et al., 1993b, 1994; Dube et al., 1994; Kalra, 1997; Kalra et al., 1999a). However, in contrast to NPY, POMC gene expression appears to be decreased in rats with diabetes or experiencing an energy deficit (Levine and Billington, 1997; Kalra et al., 1999a). Opioid peptides may provide the palatability and rewarding aspects of feeding rather than those for energy needs.

β-Endorphin-deficient mice were created by introducing a point mutation into the POMC gene that translates to a truncated prohormone lacking the entire COOH-terminal amino acid region encoding β-endorphin (Rubinstein et al., 1996). The single-copy POMC gene encodes the large precursor, which yields not only the opioid, β-endorphin, but also the nonopioid peptides, adrenocorticotropic hormone (ACTH) and α-melanocyte-stimulating hormone (α-MSH). The homozygous mice had normal birth weights and growth and development into adulthood. However, after puberty, the mice attained 10–15% greater body weight than wild-type mice. No significant changes in CRF mRNA in the PVN were reported. Mice that lack μ-receptors have been generated by several laboratories, and the main biological actions of morphine were abolished in the mutant mice, including analgesia, reward and physical dependence (Matthes et al., 1996; Kieffer, 1999). κ-Opioid deficient mice were reported to have a modified nociceptive threshold in response to visceral pain (Simonin et al., 1998). However, only limited information is available on feeding and body weight regulation in these mutant mice as in the mice lacking β-endorphin. Normal and larger litter size (but no difference in body weight) are reported in the μ- and κ-receptor deficient mice, respectively. No apparent compensatory changes in the expression of the opioid peptides or the remaining opioid receptor subtypes have been noted (Kieffer, 1999). Research is warranted on the role of each opioid receptor in palatability (Levine and Billington, 1997), as well as the inactivation of all components of the opioid system.

6. AGRP. AGRP is a recently discovered 132-amino acid peptide that has generated intense interest because a growing body of evidence indicates it has a major role in the regulation of feeding and body weight (Ollmann et al., 1997; Shutter et al., 1997; Wilson et al., 1999). AGRP was identified by virtue of its sequence similarity to the product of the Agouti coat color gene, a paracrine-signaling molecule produced normally in the skin that inhibits the effect of α-MSH, a pigment factor, on MC-1 receptor (Bultman et al., 1992; Lu et al., 1994; Leibel et al., 1997). Instead of being expressed only at a certain time during hair growth, Agouti is constitutely expressed throughout the body of yellow Agouti (A^y) mice, and this ectopic Agouti expression gives rise to pleiotropic effects including yellow coat color, obesity, insulin resistance, hyperglycemia, and increased body length. The dominant obesity syndrome was produced by expressing wild-type Agouti cDNA under the control of a ubiquitous promoter such as β-actin in transgenic mice (Klebig et al., 1995; Ollmann et al., 1997). Because mice homozygous for null mutations of Agouti do not display abnormalities of weight regulation (Wilson et al., 1999) and because ubiquitous overexpression of AGRP in transgenic mice recapitulates the increased body weight gain and body length phenotype, obesity and diabetes caused by ectopic Agouti expression occurring naturally in the yellow mice or by transgenic technology are likely explained by the ability of Agouti to mimic AGRP (Graham et al., 1997; Ollmann et al., 1997).

AGRP is expressed only in the ARC of the hypothalamus in the brain, and all of the AGRP-producing neurons are NPY-positive and project to various hypothalamic (such as PVN and DMH) and extrahypothalamic sites (Broberger et al., 1998a,b; Elias et al., 1998; Hahn et al., 1998; Haskell-Luevano et al., 1999). Like NPY, expression of AGRP is up-regulated in leptin deficiency due to fasting or mutation (Ollmann et al., 1997; Shutter et al., 1997; Hahn et al., 1998; Wilson et al., 1999). It is yet to be determined whether NPY and AGRP are in the same or parallel pathways for appetite regulation. AGRP is a potent and selective antagonist of MC-3 and MC-4 receptors (Yang et al., 1999), the melanocortin receptors implicated in control of energy balance (see *Section B. 1.*). The inhibition of melanocortin receptors may thus lead to the obese phenotype that is associated

with hyperphagia, decreased thermogenesis, and increased caloric efficiency (Fan et al., 1997; Miltenberger et al., 1997).

Humans also have an agouti gene that is normally expressed in adipose tissue, unlike in the mouse. To model human agouti expression, transgenic mice were generated that express murine agouti at high levels in adipose tissue under the regulatory control of the adipocyte lipid-binding protein (aP2) promoter (Mynatt et al., 1997). The aP2-agouti transgenic mice are not obese or diabetic, but combined insulin treatment promotes obesity, an implication for human obesity.

7. Other Orexigenic Signals. GABA, a predominant inhibitory transmitter in the CNS, can stimulate feeding (Morley, 1987; Kalra et al., 1999a). Central administration of the $GABA_A$ receptor agonist muscimol either i.c.v. or by microinjection into the PVN and other sites in the brain stimulated feeding, a response blocked by the specific $GABA_A$ receptor antagonist, bicuculline (Morley et al., 1981; Tsujii and Bray, 1991; Stratford and Kelley, 1997). GABA is coexpressed in an NPY-producing subpopulation of neurons in the ARC and is reported to have anatomical and functional relationships with other orexigenic signals such as galanin and β-endorphin (Blasquez et al., 1994; Horvath et al., 1997). These results suggest that GABA is a component in the interconnected orexigenic network (Pu et al., 1999b; Kalra et al., 1999a). Mice devoid of $GABA_A$ receptor $\beta 3$ subunit that is an essential component of the receptor, developed epilepsy, hypersensitive behavior, cleft palate, and a high incidence of neonatal motility (Homanics et al., 1997). The mutant mice that survived were runts until weaning but achieved normal body size by adulthood. GABA is synthesized by two isoforms of glutamic acid decarboxylase, GAD-65 and GAD-67. GAD-67 deficient mice exhibited a perinatal lethal phenotype, although GAD-65 deficient mice exhibited increased anxiety-like behaviors with normal glucose tolerance and body weight (Asada et al., 1997; Condie et al., 1997; Kash et al., 1997, 1999). Another genetic approach is needed to examine the role of GABA in appetite regulation independent from its effect on normal development

It has been shown that activation of α_2-adrenergic receptors in the PVN of the hypothalamus induces feeding whereas the perifornical region contains β-adrenoceptors that inhibit feeding (Leibowitz, 1989, 1992). The α_2-adrenergic system is a selective system for carbohydrate intake and is particularly active at the onset of the animals' active cycle. Infusions of NE into the VMH, but not the PVN, produce a sustained hyperphagia, reduced sympathetic activity, increased insulin, and obesity over a 20-day period (Shimazu et al., 1986). The α_2-adrenergic receptors include three distinct subtypes α_{2A}, α_{2B}, and α_{2C}, among which α_{2A} and α_{2C} subtypes are expressed in the CNS (MacDonald et al., 1997). Mice deficient in each of the α_2-adrenergic receptor subtypes were generated, which were viable and appeared grossly normal, although detailed analysis on feeding and body weight regulation has not been reported (Link et al., 1996; MacMillan et al., 1996; MacDonald et al., 1997).

Growth hormone-releasing hormone (GHRH) has been shown to increase feeding after central administration (Vaccarino et al., 1985; Morley, 1987; Inui et al., 1991). This action was shared by the new class of penta- or hexapeptides of growth hormone-releasing peptides (Okada et al., 1996). The feeding response to GHRH may follow as an inverted U-shaped dose-response curve with higher doses inhibiting feeding. Mouse-metallothionein-human GHRH transgenic mice were developed previously (Hammer et al., 1985). Overproduction of GHRH was observed in several tissues, including the pituitary, pancreas, and arcuate nucleus of the hypothalamus. The mice exhibited a dramatic increase in somatotrope function and an accelerated rate of growth, providing an animal model of acromegaly.

VGF is a secreted polypeptide of unknown function that is synthesized by neurons and is abundant in the hypothalamus (Levi et al., 1985; Snyder and Salton, 1998). Mice lacking VGF displayed dramatically decreased body weight and body fat, the major defect of which is due to excess energy expenditure and not to decreased food intake (Hahm et al., 1999). The mice had increased oxygen consumption at rest and increased locomotor activity despite normal sympathetic tone and somewhat reduced levels of thyroid hormone, suggesting that VGF may play a novel role in energy expenditure regulation.

B. Hypothalamic Inhibitors of Food Intake

1. MC. The MC system involves peptides that are processed from the polypeptide precursor POMC, which is produced by neurons in the ARC of the hypothalamus and the nucleus of the tractus solitarius (Adan and Gispen, 1997). Several of the peptide products of the POMC gene such as α-MSH have been implicated in the regulation of feeding behaviors (Table 3). α-MSH and the MC mimetics inhibit feeding in rats, mice, and agouti obese mice, an effect that is counteracted by MC antagonist (Fan et al., 1997). To date, five MC receptors have been characterized, of which MC-3 and MC-4 receptors are expressed in the hypothalamus of the brain (Adan and Gispen, 1997). A highly selective MC-4 receptor antagonist augments feeding in satiated animals and long-term blockade increases food intake and body weight gain leading to obesity (Kask et al., 1998a,b; Skuladottir et al., 1999). Altered energy balance causes selective changes in MC-4, but not MC-3, receptor binding in hypothalamic regions such as VMH, DMH, and ARC (Harrold et al., 1999). Some of the POMC neurons express functional long form of the leptin receptor, and both POMC mRNA levels and plasma leptin levels decrease after fasting and in the *ob*/*ob* mouse (Schwartz et al., 1997; Cheung et al., 1997; Mizuno et al., 1998). Up-regulation of MC-4 receptor binding was observed in

TABLE 3
Hypothalamic inhibitors of food intake and their effect on body weight regulation

	Amino Acid Length	Site of Production	Site of Action	Effect on Body Weight			
				Administration	Antagonist	Overexpression	Knockout
Melanocortin (α-MSH)	13	ARC	PVN	↓	↑	?	↑ (MC-4 R) ↑ (POMC)[b]
CRF[a]	41	PVN	PVN	↓	↑	↑[a]	→
Urocortin	40	LH	VMH	↓	↑	?	?
GLP-1	30	Brainstem	PVN	↓	↑	?	→ (GLP-1 R)
CART	48	ARC, DMH, PVN	PVN (?)	→	?	?	?
Neurotensin	13	ARC, DMH, PVN	PVN, VMH	→	→	?	?
Bombesin (GRP)	14 (27)	PVN	PVN	↓	→	?	↑ (BN-3 R) → (GRP R)
CCK-8	8	PVN	LH, PVN	→	→	?	→ (CCK-A R)
Serotonin	—	Brainstem	PVN, VMH	↓	↑	?	↑ (5-HT 2_c R)
Interleukin-1β	152	ARC	PVN, VMH	↓	↑	?	→

[a] CRF transgenic mice show Cushing's syndrome with elevated levels of glucocorticoids.
[b] Mice lacking POMC-derived peptides have obesity, defective adrenal development, and altered pigmentation (Yaswen et al., 1999).
Abbreviations: α-MSH, α-melanocyte-stimulating hormone; CRF, corticotropin-releasing factor; GLP-1, glucagon-like peptide-1_{7-36} amide; CART, cocaine- and amphetamine-regulated transcript; CCK-8, cholecystokinin octapeptide; MC, melanocortin; R, receptor; BN, bombesin; GRP, gastrin-releasing peptide; ARC, arcuate nucleus of the hypothalamus; PVN, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus; DMH, dorsomedial nucleus of the hypothalamus; LH, lateral hypothalamic area; ↑, increase; ↓, decrease; →, no change; ?, not reported.

such leptin-deficient food-restricted rats as well as leptin-resistant *fa/fa* Zucker rats, whereas down-regulation of the receptor binding was observed in diet-induced obese rats, probably reflecting changes in the release of endogenous ligand, α-MSH (Harrold et al., 1999).

Targeted disruption of the MC-4 receptor produced many of the hallmark features of the yellow obese syndrome without producing yellow fur (Huszar et al., 1997). In this model, the magnitude of hyperphagia and weight gain were significantly higher than that observed in agouti transgenic mice or the yellow mutant mice of the same genetic background, suggesting complete versus partial antagonism of the receptor (Miltenberger et al., 1997). MC-4 receptor-deficient mice did not respond to the anorectic actions of the cyclic MC agonist MT, a potent MC-3 and MC-4 receptor agonist, indicating that α-MSH inhibits feeding primarily by activating MC-4 receptor (Marsh et al., 1999). Obese MC-4 receptor-deficient mice were resistant to both peripherally and centrally administered leptin, although young nonobese mice showed a blunted response to feeding-inhibitory actions of leptin. It was also suggested from double-mutant studies that obesity that results from leptin deficiency is not caused by altered MC signaling because A^y was observed to have an additive effect on weight gain in adrenalectomized *ob/ob* animals (Boston et al., 1997). These data demonstrate that melanocortin signaling transduced by MC-4 receptor is not an exclusive target of leptin action and that factors resulting from obesity contribute to leptin resistance. The MC-4 receptor-deficient mice responded normally to the anorexigenic signals such as ciliary neurotrophic factor and urocortin, as well as to the orexigenic signals such as NPY and PYY, indicating that these neuromodulators may act independently or downstream of MC-4 receptor signaling (Marsh et al., 1999). However, enhanced response to CRF was observed in these mice, which may suggest compensatory changes in the absence of the MC-4 receptor signaling. Taken together, these results indicate the importance of MC signaling in the energy homeostasis equation, the regulation of which could be mediated by changes in agonist and/or antagonist levels, that is, a tonic restraint on feeding by MC through MC-4 receptor and removal of this restraint by AGRP leading to hyperphagia and obesity. NPY may be a downstream target of melanocortin signaling because aberrant expression of NPY in the DMH of the hypothalamus is observed in the models of the agouti obesity syndrome (Kesterson et al., 1997) and MC-4 receptor antagonist-induced feeding appears to be mediated by NPY Y_1 receptor (Kask et al.,1998b). This may be in keeping with the notion that the drive for food is chemically coded through the release of orexigenic signals (Morley, 1987; Kalra et al., 1999a; Inui, 1999a). The functional interrelationships between orexigenic β-endorphin and anorexigenic α-MSH needs to be clarified.

Very recently, mahogany protein was identified. It is expressed in various regions of the body, including the VMH of the hypothalamus (Gunn et al., 1999; Nagle et al., 1999). The mahogany protein is thought to be involved in the agouti pathway to compensate for agouti overexpression. This protein may act at or upstream of melanocortin receptors and may be involved in suppression of diet-induced obesity, the most common form of human obesity (Nagle et al., 1999).

2. CRF and Urocortin. CRF is a 41-amino acid mammalian neurohormone that is best known as the major physiological regulator of pituitary ACTH secretion and, in addition, stimulates complimentary stress-related endocrine, autonomic, and behavioral responses (Vale et al., 1981; Owens and Nemeroff, 1991; Turnbull and Rivier, 1997). There is considerable evidence indicating that CRF is an endogenous inhibitor of food intake. Injection of CRF into the brain, specifically into the PVN of the hypothalamus, a major site of CRF expression, decreases spontaneous feeding or fasting-induced feeding (Morley, 1987; Schwartz et al., 1995; Levine and Billington, 1997; Heinrichs et al., 1998). CRF decreases

feeding stimulated by GABA agonist (muscimol), norepinephrine, dynorphin, and NPY (Levine et al., 1983). Chronic administration of CRF causes sustained anorexia and progressive body weight loss (Schwartz et al., 1995). Central pharmacological blockade with CRF antagonists or antisense oligonucleotide, immunoneutralization, or immunotoxin targeting of CRF in the hypothalamus enhances basal and NPY-stimulating feeding, suggesting that CRF may tonically restrain the actions of orexigenic signals (Heinrichs et al., 1991; Menzaghi et al., 1993; Hulsey et al., 1995). Both CRF and NPY may exert local site-specific effects on feeding behavior within the PVN relative to the extrahypothalamic site that constitutes a sensitive substrate for nonappetite behavioral actions of these peptides (Heinrichs et al., 1998). Central CRF blockade also inhibits anorexia evoked by stress such as physical restraint or by interleukin (IL)-1, suggesting that CRF may be directly related to stress-related changes in feeding (Krahn et al., 1986; Uehara et al., 1989). CRF mediates its actions through interaction with two distinct receptor subtypes, CRF-1 and CRF-2, which have been cloned and characterized (Chalmers et al., 1996; Turnbull and Rivier, 1997). CRF-2 receptor is primarily involved in the feeding-suppressive and thermogenic response to CRF and CRF-related peptides (Martinez et al., 1998; Smagin et al., 1998). Urocortin is a 40-amino acid peptide that is a potent activator of CRF-2 rather than CRF-1 receptors (Vaughan et al., 1995; Spina et al., 1996). Urocortin reduces food intake and promotes weight loss at doses that do not activate the stress response (Spina et al., 1996; Asakawa et al., 1999). This makes urocortin all the more likely to be a regulator of energy homeostasis, although its role in appetite-regulating pathways needs to be determined (Inui, 1999a; Kalra et al., 1999a).

Previously, a transgenic mouse model of CRF overexpression was developed that exhibited an increase in anxiogenic behavior as well as a change in female sexual receptivity (Stenzel-Poore et al., 1992, 1994). The mice had elevated levels of activity in the hypothalamic-pituitary-adrenal axis and became obese, resembling Cushing's syndrome in humans. CRF-deficient mice were also developed which revealed a fatal glucocorticoid requirement for lung maturation and a deficient, sexually dimorphic adrenal response to stress and impaired adrenal rhythmicity (Muglia et al., 1995, 1997). However, they did not exhibit increased food intake or body weight under basal conditions, nor did they display smaller decreases in feeding after adrenalectomy known to upregulate CRF production and release (Jacobson, 1999). Corticosterone replacement completely blocked the adrenalectomy-induced decrease in feeding and body weight in all mice and frequently stimulated food intake in CRF-deficient mice (Jacobson, 1999). This was achieved at plasma corticosterone levels above the circadian peak, which probably occupied type 2 glucocorticoid receptors. There was no significant difference in the plasma levels of leptin and insulin between the control and the knockout mice, although the knockout mice tended to have lower insulin levels (Muglia et al., 1997). These results indicate that factors in addition to CRF are involved in controlling basal and glucocorticoid-associated effects on feeding. NPY could potentially mediate the effects of glucocorticoid on appetite in this model (Zakrzewska et al., 1999). CRF-1 receptor-deficient mice have been generated and their phenotype confirms the obligatory role of this receptor in stress-related reactions as shown by severely blunted adrenal response to stress and decreased anxiety levels (Smith et al., 1998; Timpl et al., 1998; Turnbull et al., 1999). However, resting ACTH secretion is maintained largely by arginine vasopressin-dependent mechanisms, and IL-6 may be involved in the pituitary-adrenal axis activation of inflammation in these mice, suggesting considerable plasticity in the mechanisms of important neuroendocrine response (Turnbull et al., 1999). A compensatory increase in the expression of CRF was detected within the PVN of the hypothalamus, but no detectable alteration was observed in the expression of CRF-2 receptor, CRF-binding protein, arginine vasopressin, or mineralocorticoid and glucocorticoid receptors (Smith et al., 1998; Timpl et al., 1998).

Although both CRF- and CRF-1 receptor-deficient mice appeared to have no gross disturbances in body weight regulation, the overexpression of CRF-binding protein confirmed the previous implication of CRF and urocortin in the regulation of feeding and body weight. The transgenic mice overexpressing CRF-binding protein in the pituitary under the control of the pituitary glycoprotein hormone α-subunit promoter and those overexpressing broadly in the body including the liver and brain under the control of mouse metallothionein-1 promoter, produced an altered circadian pattern of food intake and a sexually dimorphic body weight gain, respectively (Burrows et al., 1998; Lovejoy et al., 1998). These results are consistent with a previous report that increased availability of CRF/urocortin in the hypothalamus by the chronic administration of CRF 6–33, a high-affinity CRF-binding protein inhibitor, significantly decreased body weight in Zucker obese rats that normally have reduced CRF content in the hypothalamus, primarily by increasing sympathetic tone and energy expenditure (Heinrichs et al., 1996).

Almost all obesities depend on the presence of adrenal glucocorticoids and an overactivity of type II corticosteroid receptors (York and Hansen, 1998). All types of hyperphagia and obesity syndromes are reversed or prevented by adrenalectomy and can be readily restored by steroid replacement (Tempel and Leibowitz, 1994). Chronic excessive stimulation of type as well as type receptor in obesity results in increased fat storage and excessive food intake, with a strong preference for fats (Tempel and Leibowitz, 1994). The mice in which type II corticosteroid receptor antisense RNA construct was ex-

pressed primarily in neural tissue by using a human neurofilament gene promoter, paradoxically developed obesity despite clear evidence for reduced glucocorticoid receptor activity in the hypothalamus, cerebral cortex, and liver (Pépin et al., 1992). The type of obesity produced was associated with reduced food intake and oxygen consumption during the dark phase (thus an increased energetic efficiency; Richard et al., 1993).

3. GLP-1. GLP-1, is produced by differential post-translational processing of the proglucagon gene in the CNS and gut (Drucker, 1998). In the CNS, GLP-1 is predominantly synthesized in the brainstem, which projects to the hypothalamic sites such as the PVN and DMH (Shimizu et al., 1987; Kreymann et al., 1989; Larsen et al., 1997). These hypothalamic sites richly contain GLP-1 binding sites and GLP-1 receptor mRNA (Shughrue et al., 1996; Turton et al., 1996). It was recently reported that hypothalamic GLP-1 may be a physiological satiety factor (Turton et al., 1996). Administration i.c.v. of GLP-1 reduced food intake in fasted rats and hyperphagia in the obese Zucker rats (Tang-Christensen et al., 1996; Donahey et al., 1998). Repeated administration of GLP-1 reduced food intake and body weight without an apparent tachyphylaxis in response (Meeran et al., 1999). The GLP-1-receptor antagonist, exendin 9–39, stimulated feeding in satiated animals, and daily administration of exendin 9–39 augmented food intake and body weight gain. The anorectic effects of GLP-1 may be mediated through NPY signaling because GLP-1 inhibited and exendin 9–39 augmented NPY-induced feeding, respectively (Turton et al., 1996; Kalra et al., 1999a). The GLP-1 receptor antagonist also blocked the leptin-induced inhibition of food intake and body weight, indicating that the GLP-1 pathway may be one of the targets for the anorectic effects of leptin (Goldstone et al., 1997).

However, targeted disruption of the GLP-1 receptor gene in mice resulted in a phenotype with normal feeding and body weight, despite fasting hyperglycemia, abnormal glycemic excursions after glucose challenge, and reduced levels of glucose-stimulated insulin secretion (Scrocchi et al., 1996). Obesity fails to develop in these mice with aging or high-fat feeding (Scrocchi and Drucker, 1998). These results support the previous implications of an essential role of GLP-1 as an incretin that potentiates the release of insulin through glucose-dependent mechanisms, but do not support those in body weight regulation.

4. Bombesin. Bombesin is a tetradecapeptide originally purified from the skin of the European frog Bombina bombina (Taché and Brown, 1982; Spindel, 1986). The two known mammalian bombesin-like peptides are neuromedin B and gastrin-releasing peptide (GRP). In the CNS, these neuropeptides are thought to play a role in the regulation of feeding behavior, metabolism, and thermoregulation. Central administration of bombesin and bombesin-related peptides elicit suppression of food intake in a variety of species, although bombesin is more potent than either mammalian peptide (McLaughlin and Baile, 1981; Gibbs, 1985; Taylor and Garcia, 1985; Merali et al., 1993). Central administration of bombesin-receptor antagonists blocked the satiety effect of bombesin and also enhanced food intake in satiated rats (Flynn, 1993; Merali et al., 1993). Attempts to identify the relevant neural circuits, using brain microinjections and lesioning, have revealed that certain hypothalamic and hindbrain structures, such as the PVN and the nucleus tractus solitarius (NTS), are particularly sensitive to the feeding suppressant effects of bombesin (Kyrkouli et al., 1987; Flynn, 1992). Meal-related fluctuations in the release of bombesin (GRP)-like peptides were reported in the PVN of rats (Plamondon and Merali, 1994). Bombesin might mediate its feeding-suppressant effects through an interaction with CRF because CRF antagonists attenuated the satiety effects of bombesin administered centrally or peripherally (Plamondon and Merali, 1997). Animal studies also suggested that at least some of the effects of bombesin-like peptides on food intake are mediated through endogenous cholecystokinin (CCK) release, although in humans, GRP can act independently to reduce food intake (Gutzwiller et al., 1994). Because the effects of systemically administered bombesin are abolished by total neural disconnection of the gut from the brain and are attenuated by central pretreatment of bombesin antiserum or antagonists, the satiety effects of bombesin may be neurally communicated to the brain where bombesin receptors participate (Plamondon and Merali, 1997).

Molecular cloning studies have revealed the identity of three mammalian bombesin receptors: the GRP receptor, the neuromedin B receptor, and bombesin receptor subtype 3 (Battey et al., 1991; Wada et al., 1991; Fathi et al., 1993). The GRP receptor binds GRP with higher affinity than neuromedin B, and the neuromedin B receptor binds neuromedin B with higher affinity than GRP (von Schrenck et al., 1990). In contrast, bombesin receptor subtype-3 binds neither GRP nor neuromedin B with an affinity higher than in the micromolar range, suggesting that the natural ligand for this receptor remains to be determined. The GRP-and neuromedin B-receptors are widely expressed in the CNS and gastrointestinal tract, whereas bombesin receptor subtype-3 shows limited expression in the CNS, notably hypothalamic nuclei such as the PVN, ARC, and DMH (Wada et al., 1991, 1992; Fathi et al., 1993). The GRP receptor is also expressed in the hypothalamus such as in the PVN, and GRP is more potent than neuromedin B in inhibiting feeding. Although neuromedin B receptor is not highly expressed in these regions, it may participate in the control of food intake in the caudal hindbrain (Ladenheim et al., 1997). It was thus suggested that both GRP and neuromedin B receptors mediate bombesin-induced satiety response, although the exact contribu-

tion of each of the receptor subtypes is difficult to determine without highly specific and selective agonists/antagonists available for functional studies.

Recently, as a complementary approach, mice were created that have a disrupted gene for each of these bombesin receptor subtypes. GRP receptor-deficient mice grew normally and had normal body weight despite the lack of suppressive effect of bombesin on glucose intake (Hampton et al., 1998). A compensatory mechanism was suggested in these mice which had an increased sensitivity to CCK, another peptide involved in regulating individual meal size (see *Section. B. 5.*). It was indicated that bombesin-induced appetite suppression is mediated through the GRP receptor. The mice also showed hyperactivity during the dark period and abnormal social behavior (Wada et al., 1997). Neuromedin B receptor-deficient mice were responsive to the feeding-suppressive effect of GRP and were not obese, and had neither increased locomotor activity nor pronounced social responses (Ohki-Hamazaki et al., 1999), suggesting that the function of neuromedin B receptor and GRF receptor are distinct despite the colocalization of these receptors in brain areas such as isocortex, hippocampus, amygdala, hypothalamus, and brainstem (Wada et al., 1997). Compensation for neuromedin B receptor deficiency by overexpression of GRP receptor or bombesin receptor subtype-3 was not observed. Bombesin receptor subtype-3-deficient mice developed obesity, which is characterized by a reduced metabolic rate (oxygen consumption), hyperphagia, and increased feed efficiency without defective temperature regulation and physical activity (Ohki-Hamazaki et al., 1997). Hypertension, increased plasma levels of leptin, and impaired glucose and lipid metabolism were associated with the obesity of these animals. Because bombesin shows only low affinity for bombesin receptor subtype-3, it would be warranted to identify the natural ligand for this receptor and its role in the appetite regulation.

5. CCK. CCK is a peptide hormone secreted from gut endocrine cells and a neurotransmitter in the brain, which can reduce food intake (McLaughlin and Baile, 1981; Baile et al., 1986; Morley, 1987; Gibbs and Smith, 1992: Crawley and Corwin, 1994; Smith and Gibbs, 1994; Schick et al., 1994). CCK exists in multiple molecular forms in the circulation and the brain, ranging from 58 to 39, 33, 22, 8, and 4 amino acids in length, although neither CCK-33 nor CCK-39 has been isolated from brain. The COOH-terminal octapeptide CCK-8 is well conserved between species and is the smallest form that retains the full range of biological activities (Inui et al., 1989; Hirosue et al., 1993). CCK stimulates receptors in the pyrolic sphincter and the afferent limb of the abdominal vagus nerve, which in turn relays a message to the brainstem and the PVN of the hypothalamus where CCK mRNA is also present (Morley, 1987; Ingram et al., 1989; Gibbs and Smith, 1992; Smith and Gibbs, 1994). CCK is also released from hypothalamic sites after intragastric nutrients and has a definite neuronal influence on food intake in the CNS (Baile and Della-Fera, 1985; Schick et al., 1986, 1987). CCK reduces and CCK antagonists increase feeding after administration into the cerebral ventricle (Baile et al., 1986; Inui et al., 1987, 1989; Hirosue et al., 1993). Mapping of CCK-sensitive brain sites in the rat revealed that active sites lie not only in the LH, but also in the medial pons and lateral medulla in the vicinity of the NTS, where vagal afferent fibers terminate (Schick et al., 1994).

Subsequent analyses with highly specific and selective antagonists have demonstrated that CCK-induced satiety is mediated by the CCK-A receptor, but not by the other known CCK receptor subtype, CCK-B/gastrin receptor (Lotti et al., 1987; Lotti and Chang, 1989; Crawley and Corwin, 1994). Although CCK-B receptor is a major receptor in the brain, CCK-A receptor mRNA is also present in localized areas of the brain including the PVN of the hypothalamus (Honda et al., 1993). A large body of evidence indicates that the CCK-A receptor-specific antagonist L-364,718 (MK-329, devazepide) blocks the inhibition of food intake by exogenously administered CCK-8, and administration of L-364,718 to mice, rats, pigs, or monkeys increases food intake (Moran et al., 1992, 1993; Hirosue et al., 1993; Crawley and Corwin, 1994). Administration of the CCK-A antagonist into the PVN decreased the satiating effect of peripheral CCK-8, suggesting that CCK released from central neurons could participate in the processing of the information produced by peripheral CCK-8 (Smith and Gibbs, 1994). However, the CCK-B receptor antagonist, L-365,260, also stimulated food intake and delayed the postprandial satiety sequence in partially satiated rats (Dourish et al., 1989). L-365,260 increased food intake after injection into the LH of rats, with limited duration of action (Schick et al., 1994). These results indicate that under certain conditions the CCK-B receptor may have a role in regulating feeding. The inability of CCK to reduce food intake over an extended period has been interpreted to indicate that it is a meal-related signal and does not have a role in the regulation of body adiposity.

Recently, it was demonstrated that exogenous CCK-8 rapidly mobilizes gastric leptin with a concomitant increase in plasma leptin concentrations (Bado et al., 1998). Evidence was provided for a synergistic interaction between leptin and CCK leading to short-term reduction in food intake (Barrachina et al., 1997). The food-reducing effect of leptin injected with CCK was specific and not associated with alterations in gastric emptying or locomotor behavior. The leptin-CCK interaction was blocked by systemic capsaicin and L-364,718 but not by L-365,260, suggesting an involvement of CCK-A receptors in the vagal afferents. It was further suggested that CCK can contribute to the long-term control of feeding and body weight when central leptin levels are elevated (Matson and Ritter, 1999). The availability of mice lacking either CCK-A or CCK-B/gastrin

receptors provided an alternative approach to further address these issues. It was clearly shown that CCK-A, but not CCK-B, receptors are involved in mediating the satiety effect of exogenous CCK (Kopin et al., 1999). Administration i.p. of CCK-8 failed to decrease food intake in mice lacking CCK-A receptors, whereas CCK-8 decreased food intake by up to 90% in both wild-type and CCK-B receptor-deficient mice. However, CCK-A receptor-deficient mice showed normal day- and night-food intake and body weight that is comparable to the corresponding age- and sex-matched wild-type controls (Kopin et al., 1999). The similarity in weights persisted throughout the rapid growth phase and extended well into adulthood. CCK-B receptor deficient mice also showed normal body weight but have a remarkable atrophy of the gastric mucosa demonstrating its importance in maintaining the normal cellular composition and function of the gastric mucosa (Nagata et al., 1996; Langhans et al., 1997). The loss of a functional CCK-B receptor did not affect the expression of CCK-A receptor mRNA in brain areas such as the cerebral cortex (Nagata et al., 1996). The role of CCK-A receptor in the control of satiety and body weight should be further examined in knockout mice because congenital mutation in the CCK-A receptor gene may be associated with obesity in humans and rats (Miller et al., 1995; Moran et al., 1998).

6. Serotonin. Serotonin (5-HT) originates from the midbrain dorsal raphe nucleus and projects to the hypothalamus, including the PVN and the VMH. It is an important modulator of many developmental, behavioral, and physiological processes, including sleep, appetite, temperature regulation, pain perception, and motor activity (Vanhoutte et al., 1993). Intense interest has been focused on understanding the role of serotonin in clinical conditions such as depression, alcoholism, and drug abuse, as well as obsessive-compulsive disorder and anxiety disorders (Leibowitz, 1992; Wurtman and Wurtman, 1995; Ramamoorthy and Blakely, 1999). Serotonin and its agonists inhibit food intake when administered either peripherally or centrally in freely feeding or food-deprived animals (Leibowitz, 1989; Sahu and Kalra, 1993; Blundell et al., 1995; Simansky, 1996; Curzon et al., 1997; Heinrichs et al., 1998). Stimulants of this monoamine reduce weight gain and increase energy expenditure in both animals and humans. Cannula mapping and lesion studies indicate that this effect is anatomically localized to the medial hypothalamus, specifically the PVN, VMH, and suprachiasmatic nuclei (Leibowitz, 1989). The serotonergic system may have a selective effect on macronutrient intake; serotonin in the PVN dose-dependently suppresses carbohydrate intake (Leibowitz, 1989; Thibault and Booth, 1999). However, several studies call into question the idea. Serotonergic drugs such as dexfenfluramine and fluoxetine can inhibit fat intake, indicating that a selective effect on carbohydrate intake may not be the most prominent feeding response to serotonergic drugs (Blundell et al., 1995; Heisler et al., 1997; Smith et al., 1998). Based on the studies in which relatively selective agonists and/or antagonists for serotonin receptor subtypes were used, it was suggested that serotonin-induced satiety was mediated by postsynaptic 5-HT_{1B} receptor sites (Leibowitz, 1989; Samanin and Garattini, 1996). Functional interrelationships between serotonin and CRF, CCK, or NPY were also suggested, which were thought to be through 5-HT_{2A}, 5-HT_{2C}, or 5-$HT_{2A(2C)}$ receptors (Samanin and Garattini, 1996; Curzon et al., 1997). However, serotonin interacts with a large number of receptors, probably up to 20 different ones (Hoyer et al., 1994; Brunner and Hen, 1997). Thus, it is difficult to assign specific functions to specific receptors by this pharmacological approach alone. There are actually no selective ligands for a number of receptor subtypes such as the 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_5, 5-HT_6, and 5-HT_7 that have been identified by molecular biological techniques (Brunner and Hen, 1997). Some of these receptors may possess a high affinity for ligands that were thought to be specific to other receptors.

Recently, 5-HT_{2C} receptor-deficient mice were generated (Tecott et al., 1995; Nonogaki et al., 1998). Initial experiments showed that mutant mice had elevated body weight (and epilepsy) and were resistant to the anorectic effect of *m*-chlorophenylpiperazine, an agonist at 5-HT_{1B} and 5-HT_{2C} receptors, indicating that 5-HT_{2C} receptors contribute substantially to the serotonergic regulation of body weight. 5-HT_{2C} receptors are known to be expressed in the PVN, LH, ARC, and DMH (Wright et al., 1995; Nonogaki et al., 1998). The obesity syndrome in the mutant mice was distinguished by its developmental pattern of physiological changes, with hyperphagia preceding the development of obesity and reduced sensitivity to leptin and insulin (Nonogaki et al., 1998). The mice thus differ from other rodent models of obesity such as *ob*/*ob* and *db*/*db* mice, which are characterized primarily by metabolic dysregulation. The 5-HT_{2C} receptor-deficient mice were more sensitive to a high-fat diet that induced obesity, and at an older age they developed insulin resistance and hyperglycemia. These results indicate a dissociation of serotonin and leptin in the regulation of feeding and indicate that a perturbation of brain serotonin systems can predispose to type 2 diabetes. In contrast, mice lacking the 5-HT_{1B} receptor developed normally and had normal food intake although they exhibited motor impulsivity, enhanced aggressive behavior, elevated alcohol consumption, and increased sensitivity to cocaine (Saudou et al., 1994; Crabbe et al., 1996; Brunner and Hen, 1997). Serotonin transporter plays a key role in the regulation of serotonergic neurotransmission by inactivating serotonin after its release into the synaptic cleft. It is the primary target for widely used serotonin reuptake-inhibiting antidepressant drugs (Ramamoorthy and Blakely, 1999). Mice lacking serotonin transporter exhibited normal

body weight although adaptive changes in serotonin synthesis, turnover, or metabolism were evidenced by the substantial depletion of neuronal serotonin and 5-hydroxyindoleacetic acid in the brain regions (Bengel et al., 1998).

7. Cytokine. Cytokines are protein molecules possessing a spectrum of biological activities that are produced by the lymphocyte and/or the monocyte/macrophage during various disease states such as infection and cancer (Plata-Salaman, 1991; Sternberg, 1997). Several proinflammatory cytokines, most notably tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-6 induce anorexia and body weight loss (Plata-Salaman, 1996; Inui, 1999c). A large body of evidence suggests that the anorexia is mediated by central neural mechanisms and that the primary targets of action reside in the hypothalamus, the area with highest densities of receptors for most cytokines (Hopkins and Rothwell, 1995). TNF-α and IL-1β produce anorexia in rats at doses estimated to yield pathophysiological concentrations in the cerebrospinal fluid of animal models or patients with wasting disorders (Sonti et al., 1996a). These cytokines are released into the circulation and are transported to the CNS through the BBB and the circumventricular organs, leaky areas in the BBB (Licinio and Wong, 1997). Peripheral cytokines can exert their effects on the brain via neural pathways or second messengers such as nitric oxide and prostanoids in the brain vasculature. Cytokines are also generated within the brain, including the hypothalamus, in response to microbial and inflammatory products or peripherally released cytokines. Cytokines such as IL-1 antagonize the NPY feeding system directly or indirectly via stimulation of leptin secretion (Grunfeld et al., 1996; Sonti et al., 1996b; Gayle et al., 1997; Sarraf et al., 1997). Conversely, leptin can increase hypothalamic levels of immunoreactive IL-1β (Luheshi et al., 1999). The leptin receptor is most closely related to glycoprotein 130, which is a common signal transducer among receptors for members of the IL-6 subfamily (Friedman, 1998; Bessesen and Faggioni, 1998). Therefore, excessive leptin or leptin-like signaling resulting from the elaboration of inflammatory cytokines in the overlapping and probably redundant cytokine network may lead to anorexia and unopposed weight loss in wasting illness (Inui, 1999c,d).

The absence of TNF-α due to targeted disruption of the gene does not prevent the development of obesity induced by a high fat or high calory diet, or gold thioglucose injection except for a small decrease in adiposity (Uysal et al., 1997; Ventre et al., 1997). Gold thioglucose causes chemical ablation of the VMH. However, a significant improvement in insulin sensitivity was observed in mice lacking TNF-α or type 1 (p55) and type 2 (p75) receptors for TNF-α, confirming previous implications of TNF-α as a mediator of insulin resistance in obesity. Normal circadian variations in food intake and body weight gain were reported in the TNF double receptor knockout mice (Leon et al., 1997). IL-1 plays a central role in local inflammatory and systemic responses after infection. The IL-1 family of proteins consists of at least three different gene products: IL-1α, IL-1β, and an IL-1 receptor antagonist (Plata-Salaman, 1991, 1996). Mice lacking IL-1β or IL-1 receptor type were normal in size, weight, or gross appearance of organs (Zheng et al., 1995; Labow et al., 1997). However, the IL-1β mutant mice resisted anorexia and fever development when challenged with turpentine, which causes localized inflammation and tissue injury (Zheng et al., 1995). IL-1 receptor antagonist overexpressing mice were created under the control of the glial fibrillary acidic protein (CNS-specific expression) or under the control of the endogenous promoter (general overexpression). Neither of these mutant mice showed any developmental or weight abnormalities (Hirsch et al., 1996; Lundkvist et al., 1999). However, IL-1 receptor antagonist null mice had reduced body weight, suggesting a physiological role of the IL-1 receptor antagonist in normal energy homeostasis (Hirsch et al., 1996). The involvement of IL-6 in the generation of fever and anorexia has also been actively studied (Plata-Salaman, 1991, 1996). Mice lacking IL-6 consumed the same quantity of food and water and had similar body weight as control mice under normal conditions (Kopf et al., 1994; Kozak et al., 1997). However, they were also resistant to fever, anorexia, and body weight loss induced by turpentine, but not by influenza and lipopolysaccharide, which both induce TNF-α (Kozak et al., 1997). IL-6-deficient mice also showed a higher degree of aggressive behavior, whereas IL-6-overexpressing mice created by the neuron-specific enolase promoter showed a tendency to be more involved in affiliative-type social interactions (Alleva et al., 1998).

8. Other Anorexigenic Signals. Thyrotropin-releasing hormone (TRH) was originally isolated from the hypothalamus. It regulates thyrotropin secretion from the anterior pituitary and possibly feeding behavior (Morley, 1987). TRH reduced both feeding and drinking after i.c.v. administration in rats (Morley, 1987; Gibbs and Smith, 1992). The TRH metabolite, cyclohistidyl proline diketopiperazine (Cyclo-His-Pro), produced a more potent and long-lasting inhibition of food intake leading to weight loss. Recent studies suggest that NPY may inhibit the activity of PVN TRH neurons, which may be a necessary component to evoke the feeding response (Diano et al., 1998). TRH is expressed in the PVN at reduced levels during fasting and is restored to normal by leptin administration (Légradi et al., 1997). TRH-deficient mice showed obvious hypothyroidism and transient growth retardation with reduced body weight gain, which was restored by T_4 replacement (Yamada et al., 1997). The mice also showed a marked decrease in insulin secretion leading to hyperglycemia, implicating a pathophysiological role of TRH in pancreatic islets.

Neurotensin is a 13-amino acid peptide that produces behaviorally specific reductions in food intake after central administration (Gibbs and Smith, 1992). Neurotensin is produced in the ARC, PVN, and DMH of the hypothalamus and its microinjection into the PVN decreases food intake (Stanley et al., 1983; Alexander et al., 1989; Kalra et al., 1999a). Leptin increases neurotensin gene expression in the hypothalamus (Sahu, 1998) and the reduced expression and/or content of neurotensin is associated with the development of obesity in *ob*/*ob* mice and *fa*/*fa* rats (Beck et al., 1989; Wilding et al., 1993). Neurotensin inhibits the orexigenic effect of MCH, but not that of NPY, after i.c.v. coadministration (Tritos et al., 1998a). A hypothalamic neuropeptide CART (cocaine- and amphetamine-regulated transcript) was recently suggested to be an endogenous satiety factor (Kristensen et al., 1998; Kuhar and Dall Vechia, 1999). The expression of CART mRNA in the ARC is decreased in food-deprived animals and is almost absent in *ob*/*ob* mice, although in the latter it is restored by leptin treatment. CART peptide is a potent inhibitor of feeding and markedly inhibits NPY- induced feeding after i.c.v. administration (Kristensen et al., 1998; Lambert et al., 1998; Kuhar and Dall Vechia, 1999). Administration of antiserum against CART increases nocturnal feeding. These results suggest that neurotensin and CART are anorexigenic signals in the brain, although genetic evidence is lacking at present.

Dopamine neural circuits have been studied intensively because of their involvement in movement control, drug abuse, and cognition. However, the role of dopamine in the central control of feeding behavior is complex and still controversial. Both dopamine-dependent anorexia and overeating are reported and may depend on behavioral processes that indirectly affect feeding (Samanin and Garattini, 1996). Any change of dopaminergic function could result in marked changes of arousal and motor behavior that make it particularly difficult to study the role of this amine in feeding. However, several neuropeptides such as galanin, CCK, and bombesin stimulate dopamine release in the nucleus accumbens (Schwartz et al., 1988; Hoebel et al., 1994, 1996). Galanin injected into the PVN evokes both feeding behavior and dopamine release in the nucleus accumbens, suggesting that dopamine is involved in reinforcing ongoing behavior, including the tendency to choose a fat-rich meal (Hoebel et al., 1994). Conditioned taste stimuli excites or inhibits dopamine release in the accumbens depending on the meaning for the animal (Hoebel et al., 1996). It is likely that different aspects of feeding such as satiety and reward are regulated by dopamine in different brain regions. Recent studies suggest that the perifornical area of the LH may be a brain region in which dopamine suppresses feeding (Leibowitz and Rossakis, 1979; Parada et al., 1988; Leibowitz, 1989). Dopamine agonists appear to affect a motivational component of feeding because appropriate doses injected in this region reduced feeding without having an effect on motor behavior (Parada et al., 1988). Dopamine D_1 receptor may be involved in this action (Samanin and Garattini, 1996) although recent molecular cloning has identified five dopamine receptor subtypes in the CNS (Civelli et al., 1993). Mice unable to synthesize dopamine specifically in dopaminergic neurons were created by inactivating the tyrosine hydroxylase (TH) gene then by restoring TH function in noradrenergic cells by using noradrenergic-specific dopamine-β-hydroxylase promoter (Zhou and Palmiter, 1995). This dopamine deficiency was not lethal to the mice, although they became hypoactive and stopped feeding a few weeks after birth. With the administration of L-dihydroxyphenylalanine, the product of TH, the mice became active and consumed more food, leading to nearly normal growth. Relatively mild feeding deficits were also observed in the mice lacking dopamine D_1 or D_2 receptors, and they grew to 70% or 85% of normal body weight, respectively (Drago et al., 1994; Xu et al., 1994; Baik et al., 1995). However, they also showed decreased rearing behavior (Drago et al., 1994), locomotor hyperactivity (Xu et al., 1994) or Parkinsons-like locomotor impairment (Baik et al., 1995). The phenotypic changes of D_5 receptor-deficient mice, if generated, may be interesting because D_5 receptor is largely confined to the limbic system and hypothalamus and is practically absent in the striatum and nucleus accumbens (Tiberi et al., 1991; Samanin and Garattini, 1996).

Pancreatic polypeptide (PP) is a 36-amino acid hormone produced within the pancreatic islets and the exocrine pancreas, and consists of PP family of regulatory peptides with NPY and PYY (Taylor, 1989; Gehlert, 1998). Previous studies demonstrated that PP administration reduces food intake, body weight, or weight gain in dogs, genetically obese mice, and patients with Prader-Willi syndrome who have hyperphagia and PP deficiencies (McLaughlin and Baile, 1981; Sun et al., 1985; Berntson et al., 1993). However, a reduction of food intake by PP is modest, and other studies failed to observe it or observed it only after high pharmacological doses (Billington et al., 1983; Taylor and Garcia, 1985). Transgenic overproduction of PP, by using the cytomegalovirus immediate early enhancer-chicken β-actin hybrid promoter, developed lean mice that had specifically reduced food intake and fat mass in body composition analysis (Ueno et al., 1999). Enterostatin is the amino-terminal pentapeptide released from pancreatic procolipase by tryptic hydrolysis after pancreatic secretion has been stimulated by the presence of fat in the duodenum (York and Lin, 1996; Erlanson-Albertsson and York, 1997). Gastric procolipase is also activated to release colipase and enterostatin (Winzell et al., 1998). After peripheral or i.c.v. administration, enterostatin selectively inhibits dietary fat intake. The peripheral effects appear to be dependent on the hepatic vagal afferents through which signals activate specific brain

nuclei, including the PVN and NTS (York and Lin, 1996). Enterostatin inhibits the κ-opioid system and it chronically reduces body weight and body fat (Erlanson-Albertsson and York, 1997). Enterostatin may be a determinant of fat appetite and the susceptibility to develop obesity on high-fat diets, although transgenic models for this peptide have not yet been reported.

C. Regulators of Thermogenesis: Sympathetic Nervous System (SNS)-UCP Axis

Activation of the SNS and the consequent releases of NE in adipose tissue leads to weight loss (Strader et al., 1998). Activation of β-adrenergic receptor in white adipose tissue stimulates lipolysis, which depletes fat stores and releases fatty acids. Activation of β-adrenoceptors in BAT increases energy expenditure via thermogenesis. BAT is a specialized form of adipose tissue that functions as a thermogenic organ in rodents (Himms-Hagen, 1992). The production of heat after SNS stimulation results in the production of uncoupling protein (UCP-1 or thermogenin). Oxidation of fuels by mitochondria produces a proton gradient across the inner mitochondrial membrane. It is the energy produced by this gradient that is used by the mitochondria to convert ADP to ATP. UCP-1 allows this proton gradient to be dissipated independent of phosphorylation and thereby uncouples fuel oxidation from ATP generation. BAT plays an important role in overall energy balance in rodents, especially in cold environments and after fluid ingestion. Recently, a number of other uncoupling proteins have been identified (Fleury et al., 1997; Gong et al., 1997; Millet et al., 1997). UCP-2 is expressed in many tissues, including white adipose tissue and skeletal muscle, the major sites considered for thermogenesis in humans. It is up-regulated in white adipose tissue in response to fat feeding (Fleury et al., 1997). A third uncoupling protein homolog, UCP-3, is preferentially and abundantly expressed in skeletal muscle in humans and rodents (Gong et al., 1997; Millet et al., 1997).

The role of brown fat in obesity has been demonstrated in mice in which expression of a transgene for diphtheria toxin under the control of a BAT-specific UCP gene promoter was used to specifically ablate BAT (Lowell et al., 1993). The mutant mice developed obesity due to decreased thermogenesis and lowered body temperature (Klaus et al., 1998). However, they were affected later by unexpected hyperphagia despite extreme hyperleptinemia (Lowell et al., 1993; Melnyk and Himms-Hagen, 1998; Mantzoros et al., 1998). The expression of NPY, AGRP, and MCH were all decreased in the hypothalamus of the mutant mice in contrast to leptin-deficient *ob*/*ob* mice, although aberrant expression of NPY was observed in the DMH of the hypothalamus (Tritos et al., 1998b). Such de novo expression of NPY was reported in other models of obesity such as Agouti, MC-4-receptor knockout mice, tubby mice, and after disruption of the VMH (Inui, 1999a,b; Kalra et al., 1999a). The hyperphagia may suggest the existence of a link between BAT function and the hypothalamic system for satiety (Mantzoros et al., 1998; Melnyk and Himms-Hagen, 1998). This is consistent with the theory that sympathetic efferent output tonically inhibits food intake (Bray and York, 1998). The BAT-deficient mice also developed profound insulin resistance and diabetes that were sensitive to diet. Mice lacking UCP-1 in BAT were cold-sensitive, indicating a defect in thermoregulation (Enerbäck et al., 1997). However, UCP-1 deficiency caused neither hyperphagia nor obesity in mice fed either a standard or a high-fat diet. This may have been due to the induction of UCP-2 in the brown fat of the mutant mice. It was reported that mice overexpressing the UCP-1 gene from the aP2 gene promoter in BAT and white adipose tissue exhibited a striking redistribution of regional fat but no change in body weight. However, they were partially resistant to obesity induced by genetic (yellow obese), dietary (high-fat), or age related factors (Kopecky et al., 1995, 1996). The potential role of glycerol-3-phosphate dehyrogenase (GPDH) in energy expenditure via futile cycling was suggested by results obtained with transgenic overexpression of GPDH gene, a gene normally expressed in BAT and other tissues (Kozak et al., 1991). In this case, mice developed BAT hypertrophy that was associated with reduced amounts of white adipose tissue.

The β_1-, β_2-, and β_3-adrenergic receptor subtypes are all involved in control of lipolysis and UCP-1 activation in rodent adipose tissue (Strader et al., 1998). β_3-Adrenoceptor is expressed primarily in adipose tissue, whereas β_1-adrenoceptor and β_2-adrenoceptor are more widely expressed. Selective β_3-adrenergic receptor agonists increased the metabolic rate, leading to weight loss and improvement in glucose tolerance in obese rodents (Lipworth, 1996). Mice lacking the β_3-adrenoceptor possess only a modest tendency to become obese relative to normal controls (Susulic et al., 1995; Revelli et al., 1997). However, there was a compensatory increase in the level of β_1- (but not β_2-) adrenergic receptor in the white adipose tissue and BAT of the knockout mice, and activation of this receptor could have prevented excessive adiposity. Because of the differences in sites of expression and pharmacology between human and rodent β_3-adrenergic receptors, mice expressing human but not murine β_3-adrenergic receptor were developed under the control of human gene regulatory elements (Ito et al., 1998). These humanized animals are useful for the development of sufficiently selective treatments for human obesity. The transgenic mice overexpressing β_1-adrenergic receptor in the adipose tissue under the control of the aP2 gene promoter were resistant to diet-induced obesity (Soloveva et al., 1997). The increased lipolytic activity observed in these mutant mice suggests that total β-adrenergic receptor activity, rather than the particular subtype, may determine overall lipolytic state. β_1-Adrenergic receptor may also be involved in the prolifera-

tion of brown fat cells (Soloveva et al., 1997). Sympathetic stimulation of protein kinase A in BAT promotes thermogenesis through UCP. Protein kinase A has two regulatory and two catalytic subunits, and the RIIβ regulatory subunit is abundant in BAT and white adipose tissue and brain, with limited expression elsewhere. Targeted disruption of the RIIβ subunit, however, resulted in lean mice with hyperphagic tendency (Cummings et al., 1996). The mice were resistant to diet-induced obesity due to chronic activation of BAT thermogenesis and elevated body temperature. Mutant BAT exhibited a compensatory increase in RIα, which almost entirely replaced lost RIIβ and elevated basal protein kinase A activity, leading to increased UCP, excess energy expenditure, and leanness. The mice that cannot synthesize norepinephrine and epinephrine by inactivating the gene encoding dopamine β-hydroxylase were hyperphagic but did not become obese because of an elevated basal metabolic rate (Thomas and Palmiter, 1997). These mice were cold-intolerant because they had impaired peripheral vasoconstriction and were unable to induce thermogenesis in BAT through UCP-1. The unexpected increase in the basal metabolic rate was not due to hyperthyroidism, compensation by UCP-1, or shivering. These results indicate that BAT has an effect on the overall metabolic rate both dependent on and independent of the SNS-UCP axis.

D. Other Regulators

Obesity may occur by preferential nutrient uptake into white adipose tissue. This was demonstrated by the specific overexpression of human glucose transporter-4 (GLUT4) in white adipose tissue by using the aP2 promoter (Shepherd et al., 1993). GLUT4 is a major facilitative glucose transporter isoform in skeletal muscle and adipose tissue (Mueckler, 1994). The transgenic mice do not exhibit hyperphagia but produce hyperplasia of adipocytes and increased fat mass, suggesting that increased uptake of glucose into white adipose tissue is sufficient to produce obesity. GLUT4-null mice had severely depleted adipose tissue and growth retardation (Katz et al., 1995). Conversely, the targeted overexpression of lipoprotein lipase (LPL) in skeletal muscle by using the muscle creatine kinase promoter and enhancer prevents diet-induced obesity by diverting lipoprotein-derived triglyceride fatty acids away from storage in adipose tissue to oxidation in muscle (Jensen et al., 1997). Acylation-stimulating protein is generated by adipocytes in the postprandial period and acts on adipose tissue to stimulate triglyceride synthesis and glucose transport (Baldo et al., 1993; Sneiderman et al., 1997). The significance of acylation-stimulating protein and other molecules involved in the metabolic pathways needs to be examined in transgenic models.

Genetic manipulation can identify previously unknown regulators of energy balance. The genes encoding receptors that mediate leukocyte adhesion were demonstrated to be involved in the regulation of adipose tissue mass. Mice deficient in intracellular adhesion molecule or its counterreceptor, leukocyte integrin αMβ2 (Mac-), developed late-onset obesity without overeating (Dong et al., 1997). Leukocyte functions may thus influence energy expenditure and/or lipid metabolism. Metallothioneins comprise a family of highly conserved metal-binding proteins that have a role in the detoxification of heavy metals as well as other functions. The metallothionein-null mice became obese at a young age and were hyperphagic in established obesity (Beattie et al., 1998). The family of basic helix-loop-helix genes comprises transcription factors involved in growth and development. Mice deficient in Nhlh2, a transcription factor made in the hypothalamus, displayed adult-onset obesity with impaired gonadal growth associated with puberty (Good et al., 1997). Decreased expression of POMC in the hypothalamus was observed in these mutant mice. Growth hormone (GH) affects carbohydrate and lipid metabolism, and GH-deficient human adults become obese and insulin resistant. Transgenic rats with low serum GH concentrations and lack of pulsatile secretion exhibit normal body growth, yet develop a type of obesity with diabetes that is responsive to stimulation of pulsatile GH secretion (Ikeda et al., 1998) The signal transducer and activator of transcription, STAT5b, has been implicated in signal transduction pathways for a number of cytokines and growth factors, including GH. STAT5b-deficient mice showed dwarfism associated with later development of obesity without substitution by the highly homologous STAT5a (Udy et al., 1997). The transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) is enriched in liver and adipose tissue, and controls the expression of a wide variety of genes coding for important metabolic pathways, including gluconeogenesis and lipid synthesis. A subset of the homozygous C/EBPβ-deficient pups die within 24 h after birth, whereas the remainder are viable, but appear to have less adipose lipid accumulation (Liu et al., 1999). Mice homozygous for deletion in the genes for both C/EBPβ and C/EBPδ had a more severe reduction in size of the white adipose tissue (Tanaka et al., 1995), and there was no up-regulation of expression C/EBPα, which is known to be required for the normal development of white adipose tissue. Sterol regulatory element-binding protein-1c is another transcription factor that has been implicated in adipocyte differentiation. Overproduction of the nuclear form of sterol regulatory element-binding protein-1c driven by the aP2 promoter developed mice with markedly decreased fat tissue and insulin resistance and diabetes mellitus (Shimomura et al., 1997, 1999). The nuclear receptors known as peroxisome proliferator-activated receptor (PPAR) family contains 3 distinct isotypes: α, β, and γ (Spiegelman and Flier, 1996). PPARα is expressed in the liver and other tissues, and controls the expression of numerous genes related to lipid metabolism. A striking metabolic defect was ob-

served in PPARα-null mice, characterized by a progressive, sexually dimorphic dyslipidemia with pronounced adiposity in females and steatosis in males (Costet et al., 1998). The mice exhibited enhanced accumulation of lipid in the liver and defective responses to fasting such as severe hypoglycemia (Kersten et al., 1999). CD36 (FAT) is a membrane protein that may have a role in the muscle uptake of long-chain fatty acids. Muscle targeted overexpression of CD36 by using the promoter of the muscle creatine kinase gene was associated with enhanced muscle fatty acid oxidation and lower body weight and fat mass (Ibrahimi et al., 1999). Protein tyrosine phosphatase 1B (PTP-1B) has been implicated in the negative regulation of insulin signaling. Disruption of the gene yielded healthy mice that had increased insulin sensitivity and obesity resistance on a high-fat diet (Elchebly et al., 1999). The disruption of the leukocyte antigen-related PTP, which has also been suggested to affect the insulin signaling cascade, produced mice with body weights that were half those of control mice in some (Ren et al., 1998), but not other (Schaapveld et al., 1997), studies.

V. Advanced Gene Targeting

There are several reasons to achieve conditional, i.e., cell-type-specific and/or inducible, gene targeting beyond classical targeted mutagenesis of the mouse germline (Rajewsky et al., 1996). Germline mutations may result in lethality, in which case there is no mouse to study gene function. Genes may exert their function at several stages of ontogeny and in different cell types. It is difficult to distinguish such spatiotemporal roles of the gene by classical gene targeting. The phenotype of transgenic mouse models may be the consequence of developmental abnormality or compensation by the related gene family, rather than the physiological role of a targeted gene product per se. Furthermore, obesity and associated physiological derangements such as diabetes are complex traits, developing later in life in most cases and involving interactions between genetic factors and acquired factors such as diminished exercise. It may be preferable to initiate a gene-targeting event in adult animals without mutation during development.

Recent sophistication of molecular genetic techniques has made it possible to circumvent such potential problems by spatiotemporally regulating a gene-targeting or transgene expression (Moller, 1994; Kühn et al., 1995; Gossen et al., 1995; Rajewsky et al., 1996, Son and Joh, 1997; Rohrer and Kobilka, 1998). The tissue-specific targeting is achieved by tissue-specific expression of *cre* recombinase enzyme, which will mediate excision of the targeted gene having *lox P* recognition sites. By this *Cre/lox P* system, the gene of interest will remain and be functional in all non-*cre*-expressing cells, but will be lost in *cre*-expressing cells. A related technique is inducible gene targeting, which enables the desired genetic change at any point in development, merely by the addition of an inducer substance, such as tetracycline binary system (Gossen et al., 1995). Although the technique has not yet been applied to a component of the feeding-regulatory cascade, *Cre/lox P* mediated, tissue-specific targeting was successfully achieved to reveal the role of insulin receptor in muscle and pancreatic β cells for fat metabolism (Brüning et al., 1998), insulin secretion (Kulkarni et al., 1999), and hippocampal *N*-methyl-D-asparate receptor for spatial memory (Tsien et al., 1996a,b). A caveat of both the tissue-specific and inducible system is the penetrance of the knockout, which may affect experimental results (Rohrer and Kobilka, 1998). This is because both systems rely on the *cre* expression and targeted excision may not occur in all cells or with the same efficiency. Differences in phenotype for transgenic mice such as D_1 dopamine receptor knockouts (Drago et al., 1994; Xu et al., 1994) may be due to mouse strain heterogeneity or differences in the methods of gene disruption (Rohrer and Kobilka, 1998). Manipulation of the gene may modify the expression of other genes located near the intended one and may thus confound the interpretation of the phenotypic changes (Olson et al., 1996). Regardless of the potential shortcomings, however, advanced gene targeting will undoubtedly be instrumental in furthering our understanding of body weight regulation.

VI. Conclusions

The application of transgenic technology to create animal models that address the pathogenesis of obesity is a recent development that is now gaining rapid momentum and receiving deserved attention (Moller, 1994; Chua and Leibel, 1997; York and Hansen, 1998; Levine and Billington, 1998). Because of the multitude and complexity of disturbances in energy intake, expenditure and partitioning that are associated with obesity, it has been difficult to determine which abnormalities are causative versus less important phenomena that are consequences of the altered neuroendocrine and metabolic milieu. Transgenic methods have provided new opportunities to modify the complex body weight regulating system and then to assess the relative importance of the individual components (Figs. 1 and 2). Once created, transgenic animal models are useful to assess the efficacy or determine the mode of action of potential new therapeutic agents.

The complicated nature of appetite and energy homeostasis regulation may be suggested from the essentially normal phenotype of NPY knockout mice (Erickson et al., 1996a), despite the potent appetite-stimulating and body weight-increasing effects of NPY observed in various animal models and species (Woods et al., 1998; Inui, 1999a; Kalra et al., 1999a). The functional discrepancy between pharmacological and genetic manipulations needs to be clarified. However, it may

include the redundancy and plasticity of the regulatory machinery for the energy homeostasis equation, a characteristic of regulated biological systems (Levine and Billington, 1998; Kalra et al., 1999a). It was reported that GRP receptor-deficient mice may have an increased sensitivity to CCK, another peptide involved in regulating individual meal size (Hampton et al., 1998). Other such examples are the UCP-1 (Enerbäck et al., 1997) and β_3-adrenergic receptor (Susulic et al., 1995) knockout mice in which UCP-2 and β_1-adrenergic receptor may substitute, respectively. Therefore, in some cases the lack of any obvious effect of a targeted mutation may not necessarily mean that the gene product is not involved in the regulation of energy homeostasis. Rather, modifying the synthesis of a particular gene at all sites and developmental stages may be a crude way of investigating its functions.

Advanced gene-targeting strategies aimed at specific alterations (on and off) of a gene product at desired tissues and times are a recent elaboration which can obviously lead to a better understanding of the role the gene product plays in body weight regulation. In conjunction with conventional pharmacological manipulations, these powerful genetic tools will provide more sophisticated animal models and unprecedented insights into the underlying mechanisms of obesity, leading to new treatments.

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