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Hypothalamic Regulatory Pathways and Potential Obesity Treatment Targets

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With an ever-growing population of obese people as well as comorbidities associated with obesity, finding effective weight loss strategies is more imperative than ever. One of the challenges in curbing the obesity crisis is designing successful strategies for long-term weight loss and weight-loss maintenance. Currently, weight-loss strategies include promotion of therapeutic lifestyle changes (diet and exercise), pharmacological therapy, and bariatric surgery. This review focuses on several pharmacological targets that activate central nervous system pathways that normally limit food intake and body weight. Though it is likely that no single therapy will prove effective for everyone, this review considers several recent pre-clinical targets, and several compounds that have been in human clinical trials.

Key Words: Obesity; hypothalamus; drug targets; melanocortin; NPY; MCH; cannabinoid; CART; CNTF; AMP kinase; topiramate.

Hypothalamic Regulatory Pathways and Obesity Targets

The increasing proportion of the population who are overweight and the health risks attributable to being overweight has impelled the search for ways to reduce body weight. One way to reduce body weight may be to exogenously activate central nervous system pathways that normally limit food intake and body weight. In the past decade, our understanding of how signals of energy status reach the brain, and the central pathways engaged by those signals, has grown dramatically. There appear to be two major modes by which the brain is informed of energy status: direct neural and humoral inputs to the brainstem, and humoral inputs to the basal hypothalamus. The brainstem is quite capable of regulating inter- and intrameal behavior, but it seems that hypothalamic control is necessary for adequate long-term energy balance. Although many potential targets are currently under investigation, the current review highlights the mechanistic rationale for targeting several key players in hypothalamic circuits regulating energy balance. We have chosen not to review the recent described effects of GLP-1 analogs and amylin because of space constraints, as well as a lack of clearly understood central mechanisms of action.

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Melanocortin System in Energy Balance

Current literature indicates the importance of the hypothalamic melanocortin system for the regulation of energy balance *(53,217)*. The melanocortin system is comprised of three components: *(1)* the melanocortin peptides alpha-, beta-, and gamma-melanocyte-stimulating hormones (α-, β-, γ-MSH) and adrenocorticotropic hormone (ACTH); *(2)* the endogenous melanocortin antagonists agouti and agouti-related protein (AGRP); and *(3)* the melanocortin receptors MC1R–MC5R.

The melanocortin peptides are posttranslational products of the proopiomelanocortin (POMC) prohormone. Tissue-specific cleavage of POMC by prohormone convertases PC1 or PC2 produces ACTH in the anterior pituitary and α-MSH in the intermediate pituitary, respectively. The POMC gene is expressed primarily in the pituitary and in two distinct regions, the arcuate nucleus of the hypothalamus (ARH) and the nucleus of the solitary tract (NTS) of the brainstem *(31, 126,190)*. In particular, the ARH has been linked with long-term mediation of body weight regulation and is responsive to leptin *(57,76)*, while the NTS is more involved with the regulation of meal initiation and termination *(101)*. The ARH also contains a subset of neurons that coexpress neuropeptide Y (NPY) and AgRP. POMC neurons in the ARH and NTS and their downstream target neurons expressing MC3R and MC4R encompass the complex melanocortin circuitry that modulates energy balance.

Both POMC neurons *(44,90)* and NPY/AgRP neurons *(256)* in the ARH respond to leptin via leptin receptors expressed on their surface. The NPY/AgRP neurons project to POMC neurons in the ARH, creating a neuronal circuit that is responsive to the regulatory effects of leptin *(57)*. Electrophysiological recordings from hypothalamic slice preparations from mice expressing POMC-GFP or NPY-sapphire have enabled characterization of these cell types

in response to leptin and other neuromodulatory compounds *(57,58,109,120,204)*. In the model proposed by Cowley et al. *(57)*, leptin hyperpolarizes NPY/AgRP neurons, inhibiting GABA release from NPY terminals, resulting in disinhibition of POMC neurons. The same study also demonstrated that the MC3R is an inhibitory autoreceptor on the POMC circuit *(57)*. Activation of POMC neurons decreases food intake and increases energy expenditure, while activation of NPY/AgRP neurons increases food intake and decreases energy expenditure *(50,217)*.

Deletion of the POMC gene in mice causes hyperphagia and obesity *(265)*. Mice that lack POMC-derived peptides (POMC–/–) *(37)* exhibit increased food intake, obesity, altered pigmentation, and adrenal insufficiency. These mice also have a reduced metabolic rate, contributing to the obese phenotype. Mice heterozygous for a null mutation in the POMC allele maintain comparable weights to wild-type mice under standard chow feeding but become obese with high-fat feeding due to increased food intake (40% more than wild type mice) *(50)*. Obesity that arises from POMC haploinsufficiency is relevant, considering recent evidence that points to a region of human chromosome 2 containing the POMC gene as a causative site for human obesity *(52)*. Null mutations in human POMC gene also lead to obesity, ACTH insufficiency, and red hair (due to lack of α-MSH in the skin) *(149,150)*. Screening of the coding region of the POMC gene in patients with a history of childhood obesity revealed a missense mutation (Arg236Gly) *(180)*. This mutation disrupts processing between β-MSH and β-endorphin, resulting in a fusion protein that disrupts central melanocortin signaling. Although human POMC gene mutations support its role in the regulation of energy balance, currently there are no clinical investigations utilizing POMC promoters as an agent to modulate energy balance.

Melanocortin Receptors

Five subtypes of G protein–coupled melanocortin receptors, MC1R–MC5R, have been cloned and characterized *(3,4,45,94,182,205)*. MC1R is expressed peripherally in melanocytes and is involved in skin pigmentation. MC2R, expressed in the adrenal cortex, responds to ACTH. MC3R and MC4R are mainly expressed in the brain and are primarily known to regulate energy homeostasis *(94,160)*. Lastly, MC5R is expressed peripherally and regulates exocrine gland secretion.

Currently, data from animal and human studies support a much stronger role for MC4R than MC3R in the role of feeding and body weight. The endogenous role of MC4R in the regulation of energy balance is illustrated by MC4R knockout mice and by the discovery of missense mutations in humans. Mice lacking MC4 receptors are obese, hyperphagic, and exhibit high plasma insulin concentrations *(32,42, 119,169,227)*. On the other hand, mice lacking MC3R demonstrate only a small increase in fat mass *(41)*. In humans, missense mutations in the MC4R have been identified *(114)* and implicated as an underlying factor in a distinct population of obese pediatric patients (4–5% of morbid obesity) *(87,88,266)*. Although MC4R mutations account for only a small percentage of obesity in developed countries, it is clear that MC4R is a critical determinant of body weight in humans, and at 4–5%, MC4R mutations are the most common genetic disorder yet described in humans.

Central administration of a potent MC4R receptor agonist, MTII, reduces food intake and body weight in rodents *(20,86,233)*. MTII injections directly into the paraventricular hypothalamus (PVH) reduce feeding, indicating that the PVH is a critical site for melanocortin regulation of food intake and energy balance *(56,98,257)*. When MTII is administered via an osmotic minipump into diet-induced obese rats, body fat decreases, indicating that the melanocortin system may be involved in regulating total body adiposity *(216)*. Conversely, intracerebroventricular (ICV) administration of HS014, a selective antagonist at MC4R, increases food intake for up to 4 h posttreatment in rats *(136)*. When an MC4R antagonist with higher affinity for MC4R (HS028) is administered intravenously, increased food intake and body weight persist for 1 wk *(224)*. MC4R plays an important role as a downstream effector in leptin's effects on food intake. Administration of an MC4R antagonist, SHU9119, blocks leptin-induced inhibition of food intake *(59,218)*, as well as leptin's metabolic effects *(59)*. In mice with congenital absence of leptin or MC4R, an additive effect on fat mass was found *(235)*; similar effects were seen in double mutant A^y and leptin-deficient mice *(26)*. The critical role of POMC neurons in leptin's regulation of body weight was illustrated in the mice lacking leptin receptors only in POMC neurons are mildly obese, hyperleptinemic, and have altered expression of hypothalamic neuropeptides *(13)*.

Therapeutic Melanocortins

Palatin Technologies has recently released data from preclinical trials with PT-15, a selective MC4R agonist, in development for the treatment of obesity. Daily food consumption in normal and DIO rodents was reduced by 30–54% and body weight decreased by 15% after 11 d of peripheral treatment with PT-15. Blood glucose, plasma insulin levels, and adipose depots were all decreased. No adverse effects have been reported with PT-15 treatment. In addition, penile erectile activity was not observed, as has been noted with other melanocortin agonists *(189)*. The reason for this difference is not yet clear. To date, no reports of human clinical trials have been reported, although Palatin Technologies has melanocortin compounds in human trials for sexual dysfunction *(189)*.

Cocaine- and Amphetamine-Regulated Transcript

Cocaine- and amphetamine-regulated transcript (CART) peptides are involved in a range of important physiological processes such as feeding, reward, and reinforcement

(118). CART mRNA was first identified as an mRNA that was upregulated in the nucleus accumbens in response to acute administration of psychostimulant drugs (cocaine and amphetamine) *(73)*. However, CART is an inappropriate name because physiological studies do not support the assumption that it is a downstream mediator of amphetamine exposure *(15)*. CART peptides represent targets of interest for the treatment of obesity, but a major obstacle in developing pharmacotherapies is the absence of identified receptors. In seeking to understand the pharmacology of CART, a secondary challenge is the difficulty inherent in handling the active peptide *(117)*. The rodent CART peptide consists of either 102 (long form) or 89 (short form) amino acid residues *(73)*. Only the short form exists in humans. In vivo, CART may be posttranslationally processed into several biologically active fragments *(151,234)*. Amino acid homology between rodent and human forms of the peptide is 95%. The human propeptide is processed into two fragments of known biological activity *(118)*. The structure of the biologically active peptide contain three disulfide bridges, and the disruption or absence of these bridges reduce the activity of the peptide *(117)*. In addition, there is tissue-specific processing of CART in the rat *(234)*, and different active forms seem to have differences in potency and activity *(14)*.

Distribution and Function of CART

CART mRNA or peptides are expressed in several sites —both centrally and peripherally—where they could influence feeding and body weight. CART has been detected peripherally in several locations, including the myenteric plexus of the gut *(81)* and sympathetic preganglionic neurons of the vagus nerve *(215)*. (For the functional significance of peripheral anatomical locations, see ref. *118*.) Because we are reviewing hypothalamic circuits, our focus will be on the ARH and connections to relevant CNS circuits. CART mRNA is heavily expressed in hypothalamic regions associated with feeding behavior, with dense expression in the ARH and PVH nuclei, as well as the lateral hypothalamic area (LHA) *(29,78,147)*. CART mRNA is highly expressed in the nucleus accumbens, which is a neural substrate for the expression of reward for behaviors such as food intake *(139)*. Within the ARH, CART co-localizes with the majority of POMC neurons *(78,148,243)*. POMC is a precursor of the anorexigenic (appetite-suppressing) α-melanocyte-stimulating hormone.

Modulation of CART by Hormones that Regulate Body Weight

Leptin Stimulates CART Expression in ARH

There is strong evidence that CART expression in the hypothalamus is linked to leptin. Approximately 35% of CART-containing neurons express leptin receptors (ObRb) in the ARH *(78)*. Acute leptin treatment induces the immediate early gene *c-fos* in CART-immunoreactive neurons in the retrochiasmatic area, PVH, ARH, DMH, and ventral premammillary nucleus *(78)*. In genetic alteration animal models, leptin is one of the strongest regulators of CART mRNA levels in the hypothalamus. Obese Zucker rats, which lack leptin receptors, have significantly lower levels of arcuate CART mRNA compared with lean controls. Obese mice (*ob*/*ob*), which lack functional leptin, show a similar downregulation of CART mRNA expression in the ARH, and arcuate CART levels are restored by leptin injection *(148)*. When normal rats are fasted for 24 or 48 h, levels of CART mRNA in the ARH decrease, probably due to decreased leptin levels *(148)*. In hyperleptinemia induced by adenovirus-mediated leptin gene transfer, CART expression increases in the hypothalamus *(245)*. Likewise, hyperleptinimia induced by a high-fat diet (HFD), is associated with increased CART expression in the hypothalamus *(203)*. Even under physiological conditions, there is a strong relationship between endogenous leptin and CART in the ARH *(258)*. Rats on a HFD for 5 d demonstrate a rise in leptin levels in the absence of a change in body fat. There is a positive correlation between leptin and the density of cells expressing CART mRNA in the ARH, and a less consistent and weaker relationship between leptin and CART in the PVH *(258)*. This site-specificity is consistent with investigations showing that expression of the ObRb is particularity dense in the ARH *(78)*.

Effect of CART on Feeding Behavior

CART Decreases Food Intake and Body Weight

Acute ICV administration of recombinant CART peptide decreases food intake, and inhibits starvation-induced feeding in rats *(2,148)*. Moreover, administration of antibodies against CART peptides increases feeding in normal rats *(148)*. Continuous ICV infusion of CART over 7 d dose-dependently decreases feeding and body weight during the first 4–5 d of treatment *(155)*. Similar results were obtained by Kong et al., in which twice daily intraarcuate injection of CART peptide for 7 d reduced daytime feeding but did not affect overall food intake or body weight, suggesting that longer treatments show little effect *(146)*.

In a pivotal paper, Kristensen et al. demonstrated that when NPY and CART were ICV-injected simultaneously, the orexigenic effect of NPY was suppressed by CART in a dose-dependent manner during the first hour after injection *(148)*. Mice with a targeted deletion of the *CART* gene (CART KO) become more obese when fed a HFD from weaning than wild-type littermates *(10)*. Moreover, CART KO mice develop higher body weights when fed standard laboratory food, but not until they reach 40 wk of age *(251)*. These results are in line with the anorectic properties of CART. However, because CART receptors have not been identified, we know little about the molecular mechanisms underlying CART's actions.

Mutations in the CART Gene Could Be Associated with Human Obesity

Although polymorphisms in the CART gene of obese individuals have been identified *(38,64,244,262)* only a few of them are associated with obesity. Del Giudice et al. have identified an Italian family with history of early-onset obesity. Members who are heterozygous for a missense mutation are severely obese from childhood *(64)*. When expressed in cultured cells, this mutation causes a decrease in CART peptide concentration *(72)*. In addition, six common polymorphisms have been discovered in the promoter region of the CART gene; only one of which is associated with obesity *(262)*. These data suggest that human genetic mutation may play a small role in the obesity phenotype by altering functional CART peptide levels.

Both animal models and human genetic studies have shown that CART peptides are significant regulators of body weight via both feeding behavior and control of metabolism. CART is a potential candidate for the treatment of human eating disorders *(117)*, but identification of a receptor for CART peptides has prevented development of CART agonists for the treatment of obesity. Likewise, other potential targets for research are CART promoters *(17)*. However, we need to know more about the molecular mechanisms of CART to increase our knowledge about the role of these peptides in feeding behavior and its potential role in the etiology of obesity.

Neuropeptide Y and Energy Balance

Data accumulated over the last 20 yr have strongly implicated NPY in the regulation of appetite and energy balance. Initial experiments demonstrated that central NPY administration markedly stimulated feeding *(46,158,226)*. Fasting increases NPY levels in the hypothalamus, and normal levels return after feeding *(128,208)*. Food deprivation increases NPY-like immunoreactivity *(33)* as well as NPY release in the hypothalamus *(18,208)*. Independent of its ability to induce profound hyperphagia, central NPY injection also has considerable metabolic effects, including increased morning corticosterone and basal insulin levels and increases in liver and white adipose tissue lipogenesis *(269)*. Although germ line deletion of NPY has little effect on feeding behavior *(84)*, ablation of NPY/AgRP neurons in adult mice causes a complete loss of feeding behavior *(102,164)*.

NPY and NPY receptors are densely expressed in hypothalamic regions associated with feeding *(5,89)*. Considerable effort has been expended to identify the physiological functions of five G protein–coupled NPY receptors (Y1, Y2, Y4, Y5, and Y6) known to mediate the action of NPY *(24,159)*. Obviously, the identification and subsequent antagonism of the NPY receptor subtype or subtypes implicated in feeding regulation, especially fasting-induced feeding (as occurs during dieting), is a goal of the pharmaceutical industry. While sufficient pharmacological evidence has accumulated to strongly implicate NPY Y1 and probably Y5 receptors in the regulation of food intake, the exact contribution of each, as well as the potential role of other NPY receptors (especially Y2 and Y4 receptors *(138,186,239)* is a matter of no small debate.

NPY Y5 Receptors

From pharmacological studies with NPY and receptor subtype selective agonists, the Y5 receptor emerged early as a leading candidate for a primary role in mediating food intake. Many NPY orexigenic agonists have affinity for the Y5 receptor, but not for the Y1 receptor *(116,260)*. In addition, administration of Y5 receptor antisense oligonucleotides decreases food intake and body weight in rats with a concomitant decrease in Y5 receptor expression in the ventromedial and paraventricular nuclei of the hypothalamus, key regions known to be involved in feeding regulation *(34, 214)*. However, potent selective Y5 receptor antagonists have shown no effect on food intake in satiated rats or on NPY-induced food intake *(131,193)*. Y5 receptor antagonists seem to be most effective in decreasing food intake when high doses of centrally administered NPY are used, or when evaluation of food intake is assessed at longer time periods after administration *(193)*. These data argue against the Y5 receptor having a significant role in normal feeding or the acute sensation of satiety. Supporting this interpretation, mice lacking Y5 receptors demonstrate normal food intake and metabolic rate with late-onset mild obesity secondary to hyperphagia *(170)*. Fasting-induced refeeding in Y5 receptor knockouts is comparable to that of wild-type mice *(170)*. Central NPY-induced feeding was only diminished in Y5 receptor knockouts when high concentrations of NPY were administered *(170)*. While interpretation of these results should be cautioned because the extent of compensatory mechanisms in gene knockout models is unknown, several reports have questioned the eminence of the Y5 receptor in normal food intake. In summary, the Y5 receptor might be involved in the maintenance or modulation of hyperphagia in response to high levels of NPY, while the Y1 receptor mediates immediate hyperphagia *(170,193)*.

NPY Y1 Receptors

Although it was originally thought that the Y5 receptor was the primary mediator of food intake regulation, greater understanding of the receptor properties have lead to a more nuanced understanding. Several lines of evidence suggest that Y1 receptor antagonists may prove to have greater potential as obesity therapeutics. Structurally diverse and selective Y1 receptor antagonists decrease feeding induced by either central NPY injection or by overnight food deprivation *(96,129,130,132–134,193,250)*. Although mice lacking Y1 receptors have lower daily food intakes and a decreased response to NPY-induced feeding, they are not lean. The lack of a lean phenotype may be correlated with the fact that Y1 receptor knockouts have reduced locomo-

tion and a lower metabolic rate than their wild-type counterparts. However, deprivation-induced feeding is dramatically reduced in Y1 receptor knockout mice, suggesting a role for the Y1 receptor not only in normal feeding, but also in re-feeding after deprivation *(191)*. This latter effect suggests that Y1 receptors may maintain food intake under conditions of deprivation, such as the self-imposed food restriction that occurs with dieting. Indeed, after food deprivation, Y1 receptor gene expression increases while Y5 receptor gene expression is unaffected *(261)*. To date, no studies have analyzed the effect of simultaneous deletion or blockade of both Y1 and Y5 receptors, owing to the technical difficulty in generating Y1/Y5 receptor double knockout mice. A feasible, yet potentially illuminating, experiment would be to test a selective Y1 receptor antagonist in the Y5 receptor knockout mouse or a selective Y5 receptor antagonist in the Y1 receptor knockout mouse.

Potential Problems of NPY Receptor Antagonists

Given the demonstrated role of NPY Y1 and Y5 receptors in feeding regulation, the pharmaceutical industry has been developing several NPY receptor antagonists. Recent reviews detailing current NPY receptor antagonists can be found elsewhere *(12,103,156)*. Briefly, a few of the largest hurdles in designing NPY receptor antagonists have been (1) oral bioavailability (ability to cross the blood–brain barrier); (2) receptor selectivity; (3) nontoxicity; and, (4) peripheral and central side effects. Although many of the NPY receptor antagonists have nanomolar affinity for their respective receptor subtypes, early generation antagonists were only effective when administered centrally (i.e., BIBP3226 and BIBO3304). The first reported selective and orally bioavailable Y1 receptor antagonist was J-104870. After oral administration, effective brain levels of J-104870 were achieved and rats decreased spontaneous food intake *(133)*. A second concern regarding NPY receptor antagonists has been determining that observed decreases in feeding are due to the selective inhibition of the appropriate NPY receptor. For example, studies have demonstrated that CGP71683A, a potent Y5 receptor antagonist, decreases both NPY-induced and deprivation-induced food intake *(74,137,267)*. However, CGP71683A also acts at serotonin reuptake site *(65)*, a system known to have profound effects on feeding *(232)*. Central administration of the first potent Y1 receptor antagonist, BIBP3226 *(207)*, also decreases NPY-induced feeding *(123,136)*. However, two observations caution the interpretation of whether this effect was due solely to antagonism of Y1 receptors. First, BIBP3226 induces behaviors consistent with CNS toxicity *(181)*. Second, in chromaffin cells, BIBP3226 has been shown to inhibit catecholamine secretion and activate nicotinic acetylcholine receptors *(270)*. Lastly, it is likely that the administration of NPY receptor antagonists as an obesity therapeutic will have unknown and possibly wide-ranging effects beyond changes in food intake and energy homeostasis. Although Y5 receptors are more discretely localized in the human brain *(188)*, human Y1 receptors are widely expressed outside of the nervous system in the kidney, heart, colon, lung, testes, adrenal gland, bone marrow, placenta and vascular smooth muscle *(247)*. Some reported effects of experimental NPY receptor antagonists include changes in blood pressure *(163)*, analgesia and neurogenic inflammation *(187)*, modulation of pituitary hormone secretion *(60)* and modulation of circadian rhythms *(264)*. To date, it can be said that there is no consensus on a single receptor subtype that could be considered *the* NPY "feeding" receptor, and the potential for NPY antagonists to have widespread effects on cardiovascular, sensory, a nd endocrine systems presents a fairly insurmountable barrier to the development of an NPY antagonist as an obesity therapeutic.

Melanin-Concentrating Hormone

In mammals, melanin-concentrating hormone (MCH) appears to be involved in a variety of higher functions including sensory processing, stress responses, and learning *(80)*. In addition, MCH is an important regulator of feeding behavior *(196)*. MCH is a 19-amino-acid cyclic peptide, synthesized primarily in neurons in the LHA and the zona incerta *(23)*, but in a distinct population from orexin-containing neurons *(30)*. MCH-containing neurons from the LHA project to several hypothalamic nuclei including the ARH, PVH, and ventromedial nucleus (VMH), as well as to extra-hypothalamic areas such as the parabrachial nucleus, dorsal vagal complex and sympathetic preganglionic neurons in the spinal cord *(125)*. Notably, both orexin and MCH in the LHA may be involved in the rewarding aspects of feeding, which have a powerful influence on food intake *(70,213)*. Thus, it is possible that MCH signaling from the LHA to the nucleus accumbens, a principal reward region in the brain, is involved in the hedonic aspects of feeding.

MCH binds to a G protein–coupled receptor termed either somatostatin-like (SLC-1) or MCHR1 *(39)*. Humans also express a second receptor for MCH, MCHR2, that is not present in the rodent brain *(113)*. Expression of MCHR1 is widespread in the brain. In the hypothalamus, MCHR1 is found in the VMH, ARH, and DMH *(39,111,211)*, as well as in the LHA *(111)*. In humans, MCHR2 is densely expressed in the ARH and VMH *(210)*.

Modulation of MCH and MCHR1 by Hormones That Regulate Food Intake

Leptin Inhibits MCH Expression

Leptin's direct effects on ARH neuronal activity represent key mechanisms by which leptin inhibits food intake and increases energy expenditure *(57)*. Arcuate neurons terminate within the LHA, in close proximity to MCH neurons and processes, allowing direct regulation of MCH neurons *(30,44,76,79,194,225)*. Indeed, antagonists of the NPY Y1 receptor inhibit MCH-induced feeding *(36)*. Administration

of AgRP or SHU9119, antagonists at melanocortin receptors, elevates MCH expression *(104)*. In addition, much evidence suggests that leptin directly inhibits MCH neurons. Leptin receptors are expressed on MCH neurons in the LHA *(82)*. In hypothalamic slices containing the LHA, leptin inhibits MCH expression *(16)*. In the hypothalamus of *ob*/*ob* mice, MCH mRNA and peptide levels are increased *(196)* and central leptin injection normalizes the MCH mRNA level *(208)*. When wild-type mice are fasted for more than 2 d, MCH expression rises significantly *(236)*. Similarly, MCHR1 expression increases in fasted and *ob*/*ob* mice, suggesting that the receptor is regulated by leptin rather than MCH availability *(144)*.

Effect of MCH on Energy Balance

MCH-deficient mice have lower body weights due to reduced feeding and enhanced metabolism, despite a reduction in both leptin and arcuate POMC mRNA *(221)*. Conversely, transgenic overexpression of MCH in the LHA increases body weight of mice fed on standard and high fat diets *(162)*. In wild-type mice, acute central MCH administration stimulates food intake, but only for a short time *(196,206)*. Chronic ICV infusion of MCH has little or no effect on body weight *(206)*. However, in mice maintained on a moderately HFD, chronic MCH infusion causes significant hyperphagia and increases body weight *(206)*. In addition to its effects on appetite, MCH also influences energy metabolism. MCH neurons project polysynaptically to brown adipose tissue (BAT). Central MCH infusion stimulates fat synthesis in liver and white adipose tissue, and reduces BAT function. Together, these actions may contribute to MCH-mediated obesity *(122)*.

Abbot et al. have demonstrated that MCH increases feeding following direct administration into the ARH. In hypothalamic explants, MCH stimulates the release of NPY and AgRP (orexigenic peptides) and decreases the release of α-MSH and CART (anorexigenic peptides) *(1)*. Therefore, it is possible that the orexigenic response following MCH administration into the ARH may be mediated, at least in part, by activating orexigenic NPY/AgRP neurons while concurrently inactivating anorectic POMC/CART neurons. Currently, it is not known if MCH regulates the activity of ARH neurons.

MCHR1 likely mediates the orexigenic effects of MCH in the hypothalamus, as well as other feeding-related functions, including olfaction, taste, and reward *(111,211)*. MCHR1 KO mice are lean and hyperactive, and have increased body temperature and sympathetic tone *(171)*. The role of MCHR1 in mediating the orexigenic and metabolic effects of MCH are confirmed by studies using MCHR1 agonists and antagonists. Systemic pretreatment with SNAP-7941, a selective antagonist with high affinity for MCHR1, inhibits central MCH-mediated intake of palatable food *(25)*. Chronic peripheral SNAP-7941 administration produces sustained decreases in body weight during at least 4 wk in rats on a HFD. Takekawa et al., using a novel selective MCHR1 antagonist (T-226296), demonstrated that oral administration of T-226296 almost completely suppressed MCH-induced food intake in rats *(229)*. Chronic ICV administration of a MCHR1 antagonist also reduces feeding and body weight in satiated rats *(220)*. More recently, Mashiko et al. using a MCHR antagonist highly selective for MCHR1 (and more than 1000-fold selective over human MCHR2) observed that efficacy of the MCHR antagonist is clearly different between lean and DIO mice (mice on HFD that become obese). The MCHR antagonist remarkably reduced body weight in DIO mice, whereas the decrease in body weight was minimal in lean mice *(172)*.

Antagonism of the MCHR1 is a promising approach to both decrease food intake and increase metabolism, and thus may be an effective approach for the treatment of obesity. Currently, several pharmaceutical companies (including Amgen, GlaxoSmithKline, Lilly, Neurocrine, Neurogen, Takada) are developing MCHR1 antagonists as potential antiobesity agents, but these drugs are in early phases of development. No human studies have been reported yet *(8)*.

Cannabinoid Signaling

For centuries, *Cannabis sativa* (marijuana) has been reported to increase hunger. *Cannabis* smokers often experience hunger, which users typically refer to as "the munchies." This "munchies" concept has prompted the development of drugs to combat appetite by blocking the systems activated by marijuana.

The psychoactive ingredient of marijuana, Δ-(9)-tetrahydrocannabinol (THC), exerts its effects by interacting with specific binding sites in the brain. The discovery of cannabinoid receptors *(177)* and their subsequent cloning have tremendously boosted our understanding of cannabinoid signaling. Two distinct cannabinoid receptors have been identified, CB1 and CB2 *(153,173,183)*. Recently, a third CB3 receptor has also been postulated *(275)*. While CB2 receptors are located in the periphery *(165,183)*, CB1 receptors are widely distributed in the mammalian brain. Specifically, CB1 receptors have been found in hypothalamic regions implicated in feeding regulation: the PVH, ARH, and LHA *(110,168,237)*.

Two identified endogenous cannabinoid ligands (endocannabinoids), 2-arachidonoylglycerol (2-AG) *(176,228)* and anandamide *(67)* increase appetite *(105,143,253,254)*. Cannabinoids and endocannabinoids act via G protein–coupled receptors *(153)*. CB1 activation inhibits voltage-gated L, N, and P/Q calcium channels *(95,166)* and activates K channels *(163)*. The current proposed role for endocannabinoids is that high postsynaptic activity causes the release of endocannabinoids, which retrogradely cross the synaptic cleft to activate presynaptic cannabinoid receptors, where

they decrease neurotransmitter to modify postsynaptic activity. It is difficult to imagine that this ubiquitous mechanism plays a specific role in energy balance *(69,256)*. Currently, an antagonist at the CB1 receptor (CB1R), Rimonabant (SR141716A; Accomplia®; Sanofi-Aventis), is in late-stage clinical trials as a therapy for obesity and metabolic syndrome, although the mechanism of action is not clearly known.

Cannabinoid Receptor Antagonists: Animal Studies

The first selective CB1R antagonist was discovered in the early 1990s *(202)*. In initial animal studies, peripheral or central administration of Rimonabant significantly reduced excessive consumption of palatable foods or drink in food-deprived and satiated animals, with no effect on water intake *(9,51,62,68,91,174,222)*. After a bout of food deprivation, tonic activity of endocannabinoids increases *(143)* and this may be linked to increased circulating ghrelin levels *(238)*. Rimonabant blocks the orexigenic effects of ghrelin injected into the hypothalamus *(238)*. Further evidence implicating CB1Rs in appetite regulation comes from mice lacking CB1Rs (CB1–/–) *(198)*. CB1–/– mice are resistant to diet-induced obesity, have enhanced leptin sensitivity, lower plasma insulin levels, and do not develop diet-induced insulin resistance. Following 18 h of fasting, CB1–/– mice eat less than their wild-type littermates. In addition, intraperitoneal Rimonabant fails to reduce food intake in the knockout mice *(68)*.

Physiological, pharmacological, and genetic data demonstrate that CB1R blockade is capable of preventing the development of obesity caused by a high-fat diet. However, CB1R inactivation, either by Rimonabant or in the knockout model, does not prevent mice from choosing a highly palatable diet. In fact, it has been suggested that CB1R activation is not responsible for the pleasure derived from the orosensory characteristics of palatable food *(198)*. Rather, CB1R inactivation prevents excessive eating after starvation *(198)*. DIO mice treated with CB1R antagonists have reduced adipose tissue mass, plasma leptin, and plasma total cholesterol (compared to pretreatment levels), indicating that CB1R antagonism improves key metabolic parameters associated with morbid obesity *(112,198)*. In addition, pair-feeding experiments in DIO rats show that in addition to reducing food intake, Rimonabant increases energy expenditure. It is noteworthy that repeated administration results in tolerance to the Rimonabant-induced reduction in food intake *(197)*. However, reduction in body weight is maintained long after food intake returns to control levels *(21,51,242)*.

Mechanisms of Cannabinoid Receptor Antagonist Suppression of Food Intake

Mechanisms of endocannabinoid regulation of energy balance may include modulation of food intake by a number of hypothalamic peptides in the LHA, ARH, and PVH in addition to direct effects in a peripheral bed (adipocytes) *(55)*. Co-localization of CB1Rs occurs with corticotrophin-releasing hormone (CRH), cocaine amphetamine regulated transcript, and melanin-concentrating hormone/pre-pro-orexin in the PVH, ARH, and the LHA, respectively *(55,115)*. CRH, an anorectic neuropeptide, may be under tonic inhibition by endocannabinoids, since CB1R knockout mice have an increased expression of CRH *(55)*. Moreover, the mediation of corticosterone-induced inhibition of CRH release in the PVH is probably mediated by postsynaptic endocannabinoids retrogradely inhibiting glutamatergic release from presynaptic neurons *(69)*. The significance of retrograde signaling employed by cannabinoids for modulation of energy balance is yet to be explored extensively.

There is a possible link between the endocannabinoid system and leptin in the modulation of food intake. In young ob/ob mice lacking leptin, hypothalamic 2-AG levels are elevated and these levels are normalized by peripheral intravenous leptin. Thus, hypothalamic endocannabinoids appear to be under negative control by leptin *(68)*. The exact mechanisms by which leptin reduces endocannabinoid levels remains to be elucidated. Because the appetite-suppressing effects of Rimonabant appear to be preserved in NPY null mice, NPY may not be involved in modulating cannabinoid activity *(68)*.

A likely interaction also exists between the cannabinoid and melanocortin systems. Alpha-MSH does not block feeding induced by THC but Rimonabant reduces feeding induced by JKC-362 (MC4R receptor antagonist). This result suggests that CB1R blockade of feeding seems to occur downstream of melanocortin-4 receptors *(241)*.

Endogenous cannabinoids likely act via reward pathways to stimulate feeding, and Rimonabant significantly reduces food intake induced by 2-AG injection into the nucleus accumbens shell (an area that is involved in appetite generation and food seeking behavior) *(143)*. Rimonabant may also act by activating the mesolimbic dopamine "reward" circuitry *(35)*. Endocannabinoids elevate extracellular levels of dopamine in the shell of the nucleus accumbens *(40)*. Cannabinoid CB1Rs located on afferent pathways to the ventral tegmental area have been indicated in mediating activity of mesolimbic dopamine neurons and thereby dopamine-induced reward behavior *(35,48,49)*. The reward mechanisms may further be mediated by opioid pathways. Combined administration of subanorectic doses of Rimonabant and naloxone (an opioid antagonist) reduce food intake in a synergistic fashion in rats *(142)* and in lean and DIO obese mice *(43)*.

Peripheral targets of the endocannabinoid system include adipocytes *(21,55)*, gastrointestinal tract *(100)*, and skeletal muscles *(161)*. Rimonabant increases metabolic activity in obese mice, indicated by an increase in oxygen consumption as well as an increase in glucose uptake in isolated skeletal muscle *(161)*. In addition, repeated administration of Rimonabant markedly increases white adipose tissue

adiponectin mRNA expression, which stimulates free fatty acid oxidation and decreases body weight, hyperglycemia and hyperinsulinemia *(21)*. Thus, Rimonabant's ability to reduce body weight and improve metabolic parameters may be due to improved adipocyte function. Rimonabant is likely exerting its weight loss effects through a complex interplay between central and peripheral mechanisms.

Rimonabant: Clinical Studies and Trials

The Accomplia Clinical Development is comprised of a two-part, Phase III program investigating effects of Rimonabant: (1) Four RIO (Rimonabant in Obesity) trials, consisting of RIO-Lipids, RIO-North America, RIO-Europe, and RIO-Diabetes, and (2) Three STRATUS (Studies with Rimonabant and Tobacco Use) trials, consisting of STRATUS-US, STRATUS-EU, and STRATUS-WW. In the RIO-Lipids and RIO-Diabetes study, specific groups of patients with metabolic syndrome and at high risk for cardiovascular disease were also investigated. Criteria for metabolic syndrome required the presence of three out of five factors: (1) increased abdominal obesity, as measured by waist circumference (men >102 cm, women >88 cm); (2) hypertension (systolic >130 mmHg and diastolic >85 mmHg); (3) hypertriglyceridemia (>150 mg/dL); (4) low HDL cholesterol (men <40 mg/dL; women <50 mg/dL); and (5) high fasting plasma glucose (>110 mg/dL). Although the use of the term metabolic syndrome is currently being questioned *(127)*, groups treated with 20 mg/d Rimonabant had a 40–50% decreased incidence of metabolic syndrome in the RIO-Lipids study *(212)*. In the RIO-Europe trial, Rimonabant caused more than a 10 kg reduction in overweight and obese patients on the 20 mg/d dose, compared to a 5.0 kg loss in the placebo group who lost 5.0 kg *(240)*. The authors contend that noted improvements in several metabolic variables (HDL cholesterol and triglyceride levels) were observed independent of weight loss. Rimonabant treatment was efficacious at the first year and was maintained at the end of the second year with chronic therapy. Blockade of CB1 receptor activity appears to be a promising therapeutic alternative for treatment of obesity. However, considered caution should be noted that recent work on CB1 receptor knockout mice has demonstrated significant acceleration in age-related cognitive decline and neuronal loss *(22)*.

Ciliary Neurotrophic Factor

Ciliary neurotrophic factor (CNTF) is a cytokine produced by glial cells. Originally recognized as a trophic factor for motor neurons, CNTF was serendipitously discovered to produce weight loss (10–15%) in a clinical study assessing its potential neuroprotective properties in lean patients with amyotrophic lateral sclerosis *(47)*. CNTF binds to a receptor complex consisting of a ligand-specific subunit (CNTFRα) and two signal-transducing subunits—leukemia inhibitory factor receptor (LIFR) and glycoprotein gp130β. The latter components of this tripartite complex are structurally related to the leptin receptor *(61,121,185)*. Indeed, leptin and CNTF act on class-I cytokine receptors *(219,230)*, and CNTF receptor binding leads to activation of signaling molecules similar to those activated by leptin. Both CNTF and leptin reduce food intake, raising the possibility for their use as potential pharmaceutical agents for modifying food intake and body weight *(27)*.

Given the structural similarities between leptin and CNTF receptors, initial investigations into CNTF-induced weight loss focused on potential leptin-like mechanisms. Like leptin, CNTF dose-dependently decreases body weight and food intake in mice, with a preferential loss of fat as opposed to lean body mass *(99,154,246)*. CNTF acts on receptors that have a similar distribution to leptin receptors within the ARH and PVH, key hypothalamic nuclei involved in feeding *(99,167)*. CNTF and leptin also have similar patterns of tis-11 activation, a primary response gene, in the ARH *(99)*. However, unlike leptin, CNTF (or a modified variant of CNTF) produces anorexia and weight loss in mice lacking leptin receptors (db/db), in diet-induced obese (DIO) mice [a model of obesity associated with leptin resistance *(99, 154)*], and in obese mice lacking MC4 receptors *(169)*. These results suggested that CNTF treatment should be investigated as an alternative obesity therapeutic to bypass the leptin resistance associated with obesity.

The mechanism by which CNTF promotes weight loss is not known with certainty. Weight loss induced by low doses of CNTF is not due to cachexia or muscle wasting *(154)*. Peripheral administration of interleukin-1, a cathectic cytokine capable of producing fever, anorexia, and weight loss, induces inflammation in cerebral vasculature, produces conditioned taste aversion, and increases circulating corticosteroids in mice. These responses were not observed with doses of CNTF causing comparable weight loss *(154)*. It has been demonstrated that food restriction (dieting) also increases circulating levels of corticosteroids as well hypothalamic levels of NPY and AgRP, signals that promote an increased feeding. Interestingly, decreased feeding in DIO mice treated with CNTF is not associated with an increase in NPY-immunoreactive fibers in the PVH, whereas pair-fed mice (mice food-restricted to same level as CNTF-treated mice) have an increased density of NPY-immunoreactive fibers compared to *ad libitum*–fed control mice. Thus, CNTF decreases food intake without eliciting either a stress response (increased corticosteroid levels) or an increase in feeding drive (increased hypothalamic NPY or AgRP). Perhaps predictably because NPY/AgRP levels were not increased in CNTF-treated animals, termination of CNTF treatment did not cause rebound overeating (increase in body weight to pre-treatment levels) that occurred in untreated pair-fed mice immediately when they were allowed to feed

ad libitum (154). Still, decreased feeding after termination of CNTF treatment does not last indefinitely. To determine the long-term effects of constitutively increased levels of CNTF, Prima et al. used a viral vector delivery system and found that CNTF has only an acute anorexigenic effect in rats that abates after 25 d, allowing the rats to return to the body weight of the control group. A serious cautionary note could be ascribed to the fact that constitutive expression of CNTF (and leptin and LIF) in the brain caused a state of chronic inflammation as reflected by DNA microarray analysis of hypothalamic gene expression *(195)*. These results are consistent with a recent study comparing CNS pathways engaged by leptin and CNTF. Kelly et al. examined CNS induction of several immediate early genes, as well as fever and COX-2 mRNA induction [an obligate step in fever production *(83)*] after peripheral administration of CNTF or leptin *(140)*. Two hours after intravenous CNTF administration, COX2 mRNA was associated with blood vessels throughout the brain, meninges and several circumventricular organs. In contrast, leptin administration did not raise COX2 mRNA levels *(140)*. Because another report found that CNTF failed to induce COX2 mRNA in hypothalamus *(273)*, the central inflammatory properties of CNTF may be dose-dependent *(154)*.

Independent of its effects on feeding, CNTF improves metabolic parameters in DIO and db/db mice. CNTF ameliorates the hyperinsulinemia and hyperlipidemia observed in DIO mice. This effect was not entirely dependent on the decrease in food intake, as pair-fed mice (given the same amount of food eaten by the CNTF-treated group) had comparable losses in body weight, but did not have lowered insulin or triglyceride levels *(154)*. In db/db mice, a model of type 2 diabetes lacking functional leptin receptors, CNTF-mediated weight loss was mediated not only by a decrease in food intake (as it was in ob/ob and DIO mice), but also by an increase in basal metabolic rate. Key effects of CNTF on glycemic control include a decrease in fasting insulin and glucose levels and a decrease in nonfasting glucose levels approaching levels of nondiabetic mice. CNTF also increases hepatic insulin sensitivity and alters liver enzymes, promoting lipid oxidation and reducing lipid synthesis.

Although CNTF and leptin are both anorexigenic cytokines that act on similar receptors, the mechanisms by which they decrease food intake appear to be quite distinct. In mice treated with gold-thioglucose, which presumably would destroy at least a subset of POMC neurons in the ARH, CNTF treatment decreased food intake, suggesting that CNTF's anorexigenic effect may not be mediated through arcuate circuits *(6)*.

CNTF's anorexigenic properties as well as its potential to improve the metabolic profiles of mice with obesity and type 2 diabetes, compelled Regeneron Pharmaceuticals, Inc. (Tarrytown, NY) to design a potent form of CNTF as a potential obesity therapeutic. Developed under the name of Axokine, the safety and efficacy of the subcutaneous injectable CNTF analog was tested in two Phase II studies (in both overweight and obese patients with and without type 2 diabetes) and in one Phase III study in obesity. In the first randomized, placebo-controlled, double-blind, dose-ranging multicenter clinical trial, three doses of recombinant CNTF were evaluated in 173 nondiabetic obese subjects *(85)*. All groups, including placebo, were given dietary instruction to decrease caloric intake by 500 kcal/d. Axokine was delivered by subcutaneous injection daily for 12 wk and subjects were followed every 3 mo for 1 yr. On average, subjects lost weight in all treatment groups. The treatment group on the optimal dose lost an average of 4.6 kg, compared to a nonsignificant weight gain of 0.6 kg in the placebo group. The second 3-mo Phase II study included over 100 overweight and obese type 2 diabetic subjects. Similar to the trial in nondiabetics, subjects treated with the optimal dose of Axokine lost 3.2 kg compared to 1.2 kg in the placebo group *(199)*. The 1-yr Phase III trial included over 2300 people, with one component using overweight and obese nondiabetic subjects and another component including type 2 diabetic obese subjects. On average, Axokine-treated subjects experienced greater weight loss than the control group (2.95 vs 1.13 kg) *(199)*. A small subgroup of Axokine-treated subjects (30%) lost a larger amount of weight over the course of the trial. Notably, the magnitude of weight loss is significantly related to the development of CNTF antibodies in the first months of Axokine treatment. Currently, there is no way to predict people who are unlikely to develop CNTF antibodies and would thus have the greatest potential to obtain large amounts of weight loss. Regardless, Axokine is no longer under development as an obesity therapy.

AMP-Activated Protein Kinase

All cells must maintain a high, nonequilibrium ratio of ATP to ADP to survive. The fact that the ATP:ADP ratio in cells remains relatively constant indicates that mechanisms maintaining this balance are very efficient. The key enzyme in this process is AMP-activated protein kinase (AMPK). AMPK is activated by glucose deprivation, exercise, or environmental stresses such as heat shock and hypoxia. Common among these stresses is depletion of cellular ATP. The fall in ATP concentration and subsequent rise in AMP concentration activate AMPK. Binding of AMP to a subunit of AMPK triggers phosphorylation of the enzyme. Activated AMPK switches on ATP-generating catabolic pathways and switches off ATP-requiring pathways, *ultimately* restoring the ATP:ADP ratio. This mechanism ensures a sensitive response to a small rise in AMP [see review from Hardie *(106)*]. Thus, AMPK acts as a cellular energy sensor or "fuel gauge" that monitors cellular AMP and ATP levels. Mammalian AMPK exists as a multi-subunit complex con-

taining catalytic [α], regulatory [β] and [γ] subunits *(107)*. Genes encoding subunits of AMPK have been recognized in all eukaryotes for which genome sequences have been completed, including fungi, plants, and animals *(107)*. The number of genes encoding each subunit varies from species to species.

Hypothalamic AMPK Activity Regulates Food Intake and Body Weight

Using genetically modified mice or pharmacological activation of AMPK, two groups have demonstrated that modulation of hypothalamic AMPK activity is sufficient to alter food intake in rodents *(7,178)*. Andersson et al. demonstrated that central injection of an AMPK activator (AICA riboside) significantly increases food intake in rats *(7)*. Likewise, mice that express constitutively active AMPK in the ARH, VMH, and DMH eat significantly more than mice expressing dominant negative AMPK in the medial hypothalamus *(178)*. The mechanism by which AMPK modifies food intake is likely through reciprocal regulation of arcuate NPY and AgRP peptide expression depending on the feeding state. Data suggest that high AMPK activity enhances orexigenic signals in the fasting state. Interestingly, they demonstrate that inhibition of hypothalamic AMPK activity is required for leptin's anorexic and weight loss effects and, furthermore, that lack of AMPK suppression causes leptin resistance *(178)*.

Modulation of AMPK Activity by Hormones That Regulate Food Intake

Anorexigenics Inhibit Hypothalamic AMPK Activity

AMPK was originally perceived as a regulator of cellular energy balance and most studies focused on its response to acute changes in energy levels within individual cells. Recently, it has been shown that AMPK is also regulated by hormones such as leptin, ghrelin, and adiponectin that regulate energy balance at the whole body level *(7,178,263)*.

Leptin's main action is in the hypothalamus, where it suppresses food intake. However, as well as inhibiting energy intake by promoting satiety, leptin also stimulates energy expenditure by activating fatty acid oxidation in skeletal muscle. Minokoshi et al. demonstrated that leptin activates AMPK in muscle via two distinct mechanisms: a direct effect on muscle and an indirect effect through the hypothalamic–sympathetic nervous system. Activation of AMPK in muscle by leptin phosphorylates and inhibits acetyl CoA carboxylase, resulting in potent stimulation of fatty acid oxidation in muscle *(179)*. In contrast to its action in muscle, leptin inhibits AMPK activity in the hypothalamus. Recently, two groups have reported that intraperitoneal leptin inhibits hypothalamic AMPK activity *(7)*. Central or peripheral leptin inhibits the subunit α-2AMPK activity selectively in PVH and ARH, but does not modify AMPK activity in other hypothalamic regions (VMH, DMH, LHA) *(178)*. In contrast, leptin increased STAT3 phosphorylation in all hypothalamic regions *(178)*.

Minokoshi et al. demonstrated that central injection of MTII, an agonist to MC3 and MC4 receptors and potent anorexigen, decreases AMPK activity in the PVH, whereas central AgRP administration increases AMPK activity. However, AMPK activity in PVH does not decrease in response to ip leptin in MC4R knockout mice. Thus, the decrease of AMPK activity in PVH in response to leptin is dependent on MC4R signaling *(178)*.

Insulin, another potent anorexigenic hormone, regulates food intake and body homeostasis. Like leptin, insulin decreases hypothalamic AMPK activity. However, central insulin administration reduces α-2AMPK activity by 25–40% in all hypothalamic regions. Thus, insulin's effect on AMPK activity is more widespread in the hypothalamus than the effect of leptin, which appears to be localized to the ARH and PVH *(178)*.

The brain has evolved specialized glucosensing neurons to monitor and respond to the availability of glucose. Unlike most neurons, which use glucose to fuel their metabolic demands, these specialized neurons use the products of intracellular glucose metabolism to regulate their activity and transmitter release *(157)*. Glucosensing neurons respond to and integrate a variety of hormonal, metabolic, neurotransmitter, and peptide signals involved in energy homeostasis regulation *(157)*. When extracellular glucose rises, feeding behavior is suppressed and autonomic tone is altered. Minokoshi et al. demonstrated that intraperitoneal glucose decreases catalytic [α] subunits AMPK activity (thus decreasing AMPK activity) in all hypothalamic regions *(178)*. Since systemic glucose injection causes hyperglycemia, which stimulates insulin secretion, the decrease in AMPK activity could have been in response to an insulin surge. However, central glucose administration, which would not stimulate insulin secretion, also suppresses AMPK activity in all hypothalamic regions, similar to the effects of peripheral glucose. Likewise, administration of a known inhibitor of intracellular glucose utilization (2-deoxyglucose) increases AMPK activity *(141)*. Thus, AMPK pathway appears to respond reciprocally to changes in cellular glucose.

Orexigenic Signals Stimulate Hypothalamic AMPK Activity

Ghrelin is synthesized in the stomach and stimulates food intake, acting at least in part via the same neuronal circuits involved in the response to anorexigenic neuropeptides. Whereas leptin inhibits food intake and increases energy expenditure, ghrelin produces a positive energy balance by promoting food intake and decreasing energy expenditure *(272)*. In contrast to the effects of leptin, intraperitoneal injection of ghrelin activates AMPK in the hypothalamus *(7,145)*. Endocannabinoids, acting via the presynaptic CB1 receptor in the hypothalamus, also stimulate appetite *(124)*.

Central and peripheral cannabinoid administration stimulates AMPK in the hypothalamus *(145)*. These results provide new evidence for an interaction between AMPK and the orexigenic actions of cannabinoids and ghrelin.

AMPK as a Potential Target for Obesity Therapeutic

Currently, there are no specific pharmacological central AMPK inhibitors. However, some drugs currently used in clinical therapy regulate hepatic and muscle AMPK activity. In culture, AMPK is activated in skeletal muscle and hepatic cells by two oral hypoglycemic drugs used to treat type 2 diabetes: metformin and rosiglitazone *(93,108,184)*. Some of the effects of these drugs, like decreases in hyperglycemia by an increase in muscle glucose uptake and a decrease in hepatic glucose production, are mediated by AMPK activation. Also, activation of AMPK by metformin or rosiglitazone is required for the increase in fatty acid oxidation in hepatocytes and in skeletal muscle *(93,184)*. However, the mechanism by which these drugs activate AMPK remains unknown. Alpha-lipoic acid (a powerful antioxidant used for treating diabetic neuropathy) causes profound weight loss in rodents by reducing food intake and enhancing energy expenditure *(141)*. Kim et al. have demonstrated that α-lipoic acid exerts anti-obesity effects by suppressing hypothalamic AMPK activity *(141)*.

The role of AMPK in the central regulation of energy metabolism is only beginning to be appreciated. Several physiological signals that regulate food intake modulate AMPK activity in the hypothalamus, suggesting that AMPK may be directly involved in the mechanisms by which these anorexigenic and orexigenic signals are transduced *(92)*. Once our understanding of the role of AMPK in the integration of these signals improves, the goal may be to target AMPK agents to specific areas. AMPK appears to be an excellent clinical target.

Topiramate

Topiramate (TPM) is an FDA-approved drug for epilepsy. The anticonvulsant, analgesic, and mood-stabilizing properties of TPM may be attributed to its diverse range of biochemical and pharmacological properties. TPM blocks both voltage-activated sodium *(66,175,231,259,274)* and calcium channels *(271)*, enhances γ-aminobutyric acid (GABA)–evoked currents *(223,248,249)*, inhibits kainate-evoked currents *(97)*, and inhibits carbonic anhydrase *(71)*. As evidenced by clinical trials and various animal models, TPM has potential as an obesity therapeutic.

Topiramate—Animal Studies

TPM significantly reduces weight gain in female lean and obese Zucker rats *(192)*, in male Osborne–Mendel rats *(268)*, and in leptin-deficient ob/ob mice *(152)*. In both Sprague–Dawley *(200)* and Wistar rats *(201)*, TPM also reduces fat deposition and energy balance *(200)*.

The reduced energy balance is mostly accounted for by a decrease in fat deposition in both inguinal and retroperitoneal (white) adipose tissue *(200)*. This is accompanied by decreased lipoprotein lipase (LPL) activity in both of these white adipose depots. LPL is secreted by parenchymal cells within tissues such as adipose tissue and muscle and migrates to the vascular surface where it interacts with plasma triacylglycerol-rich lipoproteins (e.g., low-density lipoprotein), leading to triacylglycerol hydrolysis *(75)*. Triacylglycerol-derived fatty acids are differentially distributed by LPL in adipose (storage) and muscle (oxidizing) tissues. Within adipose tissue, increased LPL activity is typically associated with increased fat mass and decreased LPL activity is characterized by weight loss.

TPM also enhances LPL activity in skeletal and cardiac muscle *(200)*, an observation consistent with the TPM's reported effects on energy expenditure. It has been suggested that increased LPL activity in brown adipose tissue (BAT) seen in TPM-treated female Sprague–Dawley rats could activate BAT thermogenesis *(200)*. The TPM-induced reduction in levels of circulating triacylglycerols has been attributed to increased LPL activity in oxidative BAT and muscle tissue and decreased LPL activity in white adipose depots *(200)*. Thus, it appears that TPM treatment may have profound effects on regulating lipid metabolism.

TPM cannot be classified as a typical anorectic agent because it does not decrease food intake in most animal models *(192)*. However, TPM mildly decreases food intake in obese Zucker rats *(192)*, Sprague–Dawley rats *(200)*, and ob/ob mice *(152)*. Nevertheless, this reduced food consumption was insufficient, on its own, to explain the observed weight loss.

Hormones such as leptin and insulin have important roles in the regulation of energy balance and metabolism *(217)*. Although the effects of TPM on leptin and insulin have not been formally characterized, TPM administration reduces circulating insulin *(192)* and glucose levels *(200)*, suggesting an increased sensitivity of glucose metabolism to insulin. Plasma leptin levels also decrease with TPM treatment in female rats *(201)*, perhaps due to TPM-induced reduction in fat mass *(201)*. In lean and ob/ob mice, leptin and TPM treatment have an additive effect on energy balance (i.e., increased energy expenditure) *(152)*. Combination treatment with leptin and TPM normalizes glucose levels in obese mice *(152)*. Leptin treatment in ob/ob mice does not attenuate or enhance the TPM-induced effects on energy balance *(152)*. Interaction of TPM with leptin and insulin needs further investigation.

TPM administration also produces central effects. TPM increases hypothalamic NPY mRNA levels and decreases mRNA levels of NPY Y1 and Y5 receptors, CRH, and type II glucocorticoid receptors *(268)*. Plasma corticosterone levels also decrease, indicating that TPM downregulates the hypothalamic-adrenal axis *(268)*.

Clinical Studies

Four clinical studies investigating TPM's effect on weight loss have been published *(11,19,28,252)*. All studies employed a co-interventional approach including exercise, diet, education and behavioral therapies. TPM was administered by subcutaneous injection *(11,28,252)* or by oral tablet *(19)* at various doses. After 1 yr of oral TPM treatment, the mean weight loss was 7.1 kg or 8.8% and the average dose was 129 mg/d *(19)*. Bray et al. reported a 6-mo trial with 385 obese subjects randomized to placebo or TPM treatment at several doses. Weight loss from baseline to 24 wk was significantly higher in all treatment groups, compared to placebo *(28)*. In addition, systolic and diastolic blood pressures significantly decreased. Two of the most recent multicenter trials have reported successful weight loss in obese subjects. One trial randomized 1289 obese subjects to injectable TPM at 96, 192, or 256 mg/d. Results from 854 subjects who completed the first year of the trial were reported. Subjects lost 5.3%, 7.3%, and 8% of their initial body weight (placebo-adjusted) in the 96, 192, and 256 mg/d groups, respectively. Blood pressure and glucose tolerance parameters were markedly improved in the TPM-treated groups *(252)*. Longer-term results were not reported because this study was terminated due to the sponsor's pursuit of a time-released version of the drug. The second multicenter trial enrolled 701 obese subjects who were treated with a very low calorie diet to induce an 8% loss of initial body weight; 293 subjects completed 44 wk of treatment. The TPM-treated groups (96 and 192 mg/d) lost 15.4% and 16.5%, respectively, of their baseline body weight, while the placebo group lost only 8.9% *(11)*. When adjusted for placebo-induced weight loss, the TPM-treated groups lost 6.5% and 7.6% of initial body weight at 96 and 192 mg/d, respectively. Likewise, this study was terminated prematurely to pursue a controlled-release version of the drug. The most frequently reported adverse events were paresthesia, somnolence, and difficulty with concentration, memory, and attention. Although still available, the use and development of TPM as an obesity therapeutic has been terminated thus far due to these adverse effects.

Conclusion

This review has considered several recent preclinical targets, and several compounds that have been in human clinical trials. Although these targets have shown some promise in preclinical trials, no *magic bullet* therapy has reached consumers. The work in this laboratory has largely focused on the role of the melanocortin system in energy balance, and we naturally feel a bias toward therapies that focus on this system. But, there are several caveats to consider before this review is perceived as a ringing endorsement of melanocortin therapies for obesity. First, although there is good evidence that melanocortin therapies may address homeostatic energy balance, there is not good evidence that obese people overconsume food because of a homeostatic drive. Rather, it is easier to believe that overconsumption occurs because of reward-based mechanisms: many of us can relate to the feeling of "being able to fit in just a small piece of pie." There is no clear evidence that the melanocortin system mediates reward, so this system may not be relevant to reward-mediated overconsumption. This is an appropriate point when considering any single mono-therapy. Alternatively, activating the melanocortin system may prove an effective method of inducing weight-loss, regardless of the cause of obesity.

The search for a single magic bullet for safe and effective weight loss for everyone may prove fruitless, so we must consider alternatives. Combination or serial therapy is the standard of care in many fields of medicine. Could it also be a better approach in energy balance? Combination therapy has the ability to act on different levels of the energy balance system, and possibly reduce the accommodation or adaptation that limits the magnitude and duration of current therapies. Designing trials that address combination therapy is difficult and expensive because the combination product must be tested for safety and efficacy against all monotherapies and placebo, but we expect to see more development on this front in the coming years.

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