It Takes Two to Tango: Combined Amylin/Leptin Agonism as a Potential Approach to Obesity Drug Development

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Abstract: The discovery of leptin in 1994 was a seminal event in obesity research. It helped to establish that body weight is tightly regulated by a complex neurohormonal feedback system and that obesity should be viewed as a disorder with a strong biological basis rather than simply the result of poor lifestyle choices and lack of willpower.

Leptin, secreted from adipocytes, acts as a prototypic long-term (tonic) adiposity signal. Although nonclinical and clinical studies have provided unequivocal evidence that leptin plays a unique, pivotal role in body weight regulation, efforts to develop recombinant leptin (metreleptin) as a monotherapy for obesity have proven unsuccessful. Amylin, secreted from pancreatic β-cells, fulfills the criteria for a short-term (episodic) satiety signal. The amylin analog pramlintide elicits sustained reductions in food intake and body weight in obese rodents and humans.

A translational research program aimed at elucidating the interaction between different islet-, gut-, and adipocyte-derived hormones led to the discovery that combined amylin/leptin agonism induces marked, synergistic, fat-specific weight loss in leptin-resistant diet-induced obese rodents. In obese humans, combination treatment with pramlintide/metreleptin led to an approximately 13% weight loss after 24 weeks, significantly more than after treatment with pramlintide or metreleptin alone.

Collectively, these findings suggest that combined amylin/leptin agonism may have therapeutic utility as part of an integrated, neurohormonal approach to obesity pharmacotherapy.

Key Words: weight loss, pharmacotherapy, adiposity signal, satiety signal

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Body weight is regulated by a complex neurohormonal feedback system that involves the interaction between peripheral hormonal signals with a distributed network of nuclei and pathways within the central nervous system (CNS).[1–4] The system is hardwired to achieve precise long-term balance between energy intake and energy expenditure and is characterized by multiple redundancies and counterregulatory mechanisms. From a teleological perspective, the latter exist for good reason because defense of body weight in states of starvation is critical for survival. However, in the current obesity-promoting environment of plentiful food and insufficient physical activity, these same neurohormonal defense mechanisms are disadvantageous and explain why most overweight individuals attempting to achieve sustained weight loss with diet alone are fighting a losing battle.[1–4]

To date, efforts to translate scientific advances in the neurobiological basis of body weight regulation into effective therapeutics for obesity have proven unsuccessful. Of note, because of the traditional target-driven approach to drug discovery, many hormonal signaling pathways—including leptin—have hitherto been studied in relative isolation. Given the multitude of redundancies and complexities in the system, it is perhaps not surprising that no single hormonal pathway has yielded the proverbial "magic bullet."

In this brief review, we describe a novel, more integrated, neurohormonal approach to obesity drug development. By combining peptide/protein hormones with different physiological roles and complementary mechanisms of action, this approach aims to harness naturally occurring synergies and overcome or mitigate redundancies, with the ultimate goal of achieving more pronounced and sustained weight loss.

First, we provide a high-level overview of the distributed network of central pathways and peripheral signals important in regulation of body weight, focusing on those elements most pertinent to our therapeutic approach, as well as the counterregulatory systems that act to defend body weight in response to energy restriction. Next, we review the physiology of 2 hormones with important roles in the regulation of body weight and food intake—leptin, the prototype adipokine, and amylin, a pancreatic β-cell hormone cosecreted with insulin. We then present data from nonclinical studies demonstrating that combination treatment with these 2 hormones in diet-induced obese rodents is able to induce marked, synergistic weight loss, including data on potential mechanisms of action. Finally, we summarize positive proof-of-concept data from a translational clinical research study with administration of analogs of these 2 hormones (pramlintide and metreleptin) in overweight/obese humans.

NEUROHORMONAL FEEDBACK REGULATION OF BODY WEIGHT

Central Nervous System

Although the hypothalamus has long been viewed as the predominant brain region governing food intake and body weight, it is now evident that regulation of energy balance is accomplished by a distributed network of nuclei across the CNS, including key areas in the hypothalamus, brainstem, and corticolimbic system (Fig. 1). These nuclei receive and integrate inputs from peripheral signals that inform the CNS of immediate and long-term changes in the nutritive state.[1–4]

The hypothalamus plays an important role in the long-term regulation of energy balance and body fat stores, as evidenced by classic lesioning experiments[5,6] as well as modern molecular

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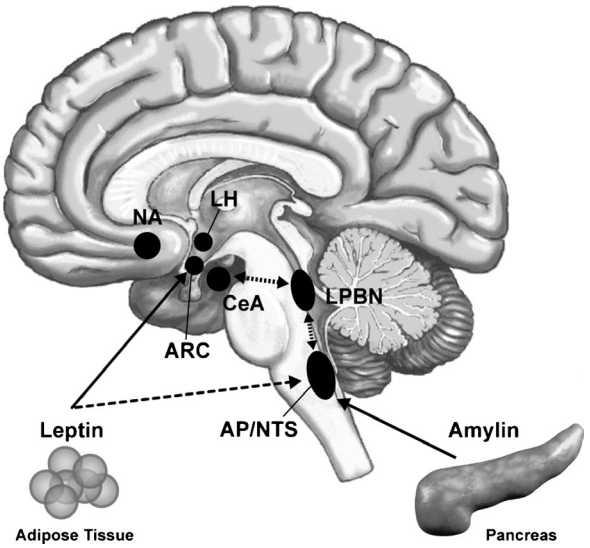

	Amylin (Pramlintide)	Leptin (Metreleptin)	Amylin/Leptin (Pramlintide/Metreleptin)
Physiology			
Origin	Pancreatic β-cell	Adipocyte	
Size	Peptide (37 aa)	Protein (146 aa)	
Physiological function	Short-term (episodic) satiety signal	Long-term (tonic) adiposity signal	
Key CNS binding sites	Hindbrain (AP/NTS)	Hypothalamus (ARC, VMH, DMH)	
Non-clinical studies in DIO rats			
Food intake	↓	↔	↓↓↓
Weight loss	↓	↔	↓↓↓
Clinical studies in obese humans			
Mechanism of action	Enhances satiety and reduces food intake	Prevents counter-regulatory responses associated with weight loss (e.g. decreases in metabolic rate, SNS tone, T3/T4 levels)	Mechanism of synergy to be determined
Weight loss	↓	↔	↓↓↓
Use in other disease states			
	Type 1 and Type 2 diabetes mellitus (with meal-time insulin)	Improvement in metabolic abnormalities with congenital leptin deficiency and severe lipodystrophy (investigational use)	

FIGURE 1. Amylin and leptin neurobiology, nonclinical, and clinical observations. SNS indicates sympathetic nervous system; T3, triiodothyronine; T4, thyroxine.

biology techniques.[7,8] The arcuate nucleus (ARC), a key site of leptin receptor signaling, contains anorexigenic preopiomelanocortin/cocaine and amphetamine-related transcript and orexigenic neuropeptide Y/agouti-related peptide neurons, which project to other important hypothalamic nuclei such as the ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), paraventricular nuclei, and lateral hypothalamus. Additional signals in these second-order projections include orexigenic factors melanin-concentrating hormone and orexin in the lateral hypothalamus and anorexigenic α-melanocyte–stimulating hormone (α-MSH) at melanocortin-4 receptors in the paraventricular nuclei and DMH.[9,10]

Hindbrain nuclei play an important role in the day-to-day control of food intake, as convincingly demonstrated by seminal experiments in decerebrated rats.[11] The area postrema (AP) and adjacent nucleus of the solitary tract (NTS), key binding sites for amylin and other circulating satiety signals, as well as recipients of vagal afferent inputs, help mediate perceptions of hunger, fullness, and satiation during and after meals. Indeed, the anorexigenic effect of several hormonal satiety signals is completely lost after lesioning of the AP.[12] Besides regulating meal size at the hindbrain level, satiety signals binding to the AP/NTS may interact with higher brain regions regulating energy homeostasis via upstream projections to the parabrachial nucleus, central nucleus of the amygdala, and hypothalamus.[12,13]

Corticolimbic regions (nucleus accumbens, amygdala, hippocampus, and sensory and orbitofrontal cortex) play an

important role in the hedonic and emotive aspects of eating.[14] As with the aforementioned brain regions, there is increasing recognition that corticolimbic regions are highly interconnected with hypothalamic and hindbrain nuclei, providing a neuroanatomical correlate for interactions between the homeostatic and hedonic control systems regulating food intake.

Peripheral Signals

To effectively function in the tight feedback control of energy homeostasis, the distributed neural network described above relies on multiple inputs from peripheral organs such as adipose tissue, the gut, and pancreatic islets. This is achieved via circulating hormones and nutrients as well as via neural (vagal) afferents. Hormones important in energy homeostasis can be categorized into long-term (tonic) adiposity signals (eg, leptin, insulin) or short-term (episodic) signals with either orexigenic (eg, ghrelin) or anorexigenic (eg, amylin, peptide YY 3–36 [PYY_{3-36}], cholecystokinin, and glucagon-like peptide-1) effects.[1–4]

These peripheral signals are integrated within the CNS to effect a variety of metabolic, hormonal, and behavioral responses, which collectively determine energy intake (eg, hunger, food seeking behavior) and energy expenditure (eg, basal metabolic rate, substrate utilization, sympathetic tone, thyroid hormone secretion). Together, peripheral and central systems interact in a complementary fashion to form a complex regulatory feedback system that is able to maintain tight control of energy balance and mount powerful counterregulatory responses to counteract weight loss.[2,4,15,16]

Neurohormonal Basis of Weight Loss Counterregulation

Restriction of energy intake (as encountered during dieting) has the immediate effect of reducing the meal-induced secretion of short-term satiety signals such as amylin and PYY_{3-36} (which are secreted in proportion to meal size). With more prolonged food restriction and loss of body fat, secretion of long-term adiposity signals (eg, leptin) decreases markedly as well. These peripheral changes trigger signaling in key hypothalamic and extrahypothalamic nuclei, with concomitant activation of orexigenic signals and inhibition of anorexigenic signals. The end result is a strong biological drive to increase energy intake, including increases in hunger and food cravings, and a host of responses to "conserve energy," including decreased energy expenditure (due largely to increased skeletal muscle efficiency), sympathetic tone, and thyroid hormone concentration.[15] Collectively, these counterregulatory responses act to limit further weight loss and eventually promote weight regain. The remarkable accuracy with which these systems can maintain body weight within a narrow "set point," despite potentially large variations in food intake and physical activity on a day-to-day basis, has important implications for the individual who is attempting weight loss. As noted, although advantageous from a teleological perspective, these counterregulatory mechanisms have the unwanted consequence of making weight loss difficult to achieve and sustain, even in the presence of excessive fat stores.

LEPTIN: A LONG-TERM (TONIC) ADIPOSITY SIGNAL

Discovered in 1994 as the missing hormone in the *ob/ob* mouse model of genetic obesity, leptin derives its name from the Greek word *leptos* for "thin," based on its striking efficacy to induce weight loss when administered to these markedly obese, leptin-deficient mice.[17] The finding that loss of a single hormone could have such a dramatic phenotype in a system filled with redundancies provided indisputable proof that leptin plays a fundamentally important role in the regulation of energy homeostasis. This is clearly the case in humans as well, in that leptin replacement (with recombinant methyonyl human leptin, metreleptin) has a profound effect to induce weight loss in leptin-deficient obese children and adults[18–20] and to improve metabolic, neuroendocrine, and immune abnormalities in states of relative leptin deficiency (see accompanying article by Bluher and Mantzoros[21]).

Individuals with general obesity have high circulating leptin concentrations.[22] Although this observation is in keeping with the role of leptin as a long-term adiposity signal, it also suggests resistance to the effect of leptin to decrease appetite and cause weight loss. Indeed, peripheral administration of leptin in rodent models of diet-induced obesity (DIO) had only marginal effects to induce weight loss,[23] and similar findings were observed in a clinical trial of metreleptin administration to obese humans.[24] With the exception of a few individuals (n = 8) assigned to a high pharmacologic dose (0.3 mg/kg), metreleptin did not induce significant weight loss, a finding confirmed in multiple subsequent phase 2 obesity studies conducted by Amgen, Inc [data on file, Amylin Pharmaceuticals, Inc]. Use of a long-acting pegylated leptin analog in obese men was similarly disappointing, with no difference in weight loss versus placebo.[25] The mechanisms underlying leptin resistance in obese individuals are not fully understood but may involve decreased transport across the blood brain barrier as well as fully downstream signaling defects that remain to be elucidated (see accompanying article by Bjorback[26]). Although it was disappointing that leptin agonism alone had no weight-lowering effect in general obesity, from a translational research perspective, it is highly reassuring that there is considerable consistency between rodent models and the corresponding human disease states.

Having established that leptin does not act as a weight-lowering, antiobesity hormone per se, subsequent studies focused on elucidating the true physiologic role of leptin. Studies in rodents[27] and, later, humans[28,29] provided evidence that leptin may act as a key hormonal mediator in weight loss adaptation and counterregulation (starvation signal). Detailed studies in humans by Rosenbaum et al.[15] in a carefully controlled clinical research environment demonstrated that leptin replacement (at doses intended to counteract the diet-induced fall of endogenous leptin concentrations) effectively prevented the decline in metabolic rate, thyroid hormone concentrations, sympathetic tone, and increases in muscle work efficiency associated with a 10% diet-induced weight loss. Thus, leptin seems to have a unique effect to prevent the counterregulatory responses associated with energy restriction, which may help mitigate the biological drive toward weight loss plateau and weight regain. In more recent studies, leptin replacement in obese individuals studied under this same paradigm also reversed changes in regional neural activity (as assessed by functional magnetic resonance imaging) associated with the weight-reduced state, such as increased activity in brain areas related to food memory and emotional responses and decreased activity in brain areas related to food sensation, restraint, and control.[16] Within this revised framework of leptin acting as a starvation signal, it seems prudent to refine the concept of leptin resistance in general obesity. That is, although obese individuals may not respond (with weight loss) to a rise in leptin (induced by administration of exogenous leptin), they seem to remain quite

sensitive to a diet-induced fall in leptin, manifesting the full spectrum of counterregulatory adaptations present in lean individuals.

AMYLIN: A SHORT-TERM (EPISODIC) SATIETY SIGNAL

Amylin is a 37–amino acid peptide hormone with both glucose-regulatory and anorexigenic actions.[29–31] Amylin and insulin are stored in the same pancreatic A-cell secretory vesicles and are cosecreted in response to food intake.[32–34] Plasma amylin concentrations rise rapidly after meals at concentrations proportional to meal size, peaking approximately 30 minutes after meal and returning to baseline after approximately 2 hours.[32,35] Fasting concentrations of amylin are also correlated with body weight and are elevated in obese individuals similar to insulin.[32,35]

Amylin acts primarily at the hindbrain, although upstream signaling to rostral CNS regions has been conclusively demonstrated.[13,36] Upon binding to specific amylin receptors within the AP, which emerge from the dimerization of the calcitonin receptor (CT[a]/CT[b]) with certain receptor activity-modifying proteins (RAMP1, RAMP2, and RAMP3),[37] amylin activates a neural circuit composed of neurons in the NTS, parabrachial nucleus, and the central nucleus of the amygdala.[38] This has the primary effect of reducing food intake at physiological concentrations, thus fulfilling the criteria of a peripheral satiety signal.[29] In rodents, administration of amylin selectively decreases intake of highly palatable foods with high fat and/or high sucrose content.[39]

Importantly, preclinical and clinical studies using administration of amylin or pramlintide (a synthetic analog of amylin), respectively, do not indicate resistance to the anorexigenic effect of amylin in general obesity. In DIO rats, peripheral amylin administration leads to sustained reductions in food intake and body weight.[39,40] Amylin-mediated weight loss was fat-specific compared with pair-fed rats that lost lean as well as fat mass.[40]

Consistent with these rodent findings, clinical studies have shown that the amylin analog pramlintide, a medication currently approved in the United States as an adjunct treatment in patients with type 1 and type 2 diabetes who use meal-time insulin therapy and have not achieved desired glucose control, reduces food intake and body weight in diabetic and nondiabetic obese humans.[41–47] A single injection of pramlintide administered 1 hour before an ad libitum buffet meal enhanced satiation (by ~58%) and reduced food intake (by ~16%) in obese subjects.[44] In a 6-week placebo-controlled study in obese subjects, pramlintide administration reduced 24-hour food intake (by ~15%–20% or ~500–750 kcal/d) as well as binge eating tendencies.[47] In a 4-month, randomized, double-blind, placebo-controlled, dose-ranging study that was followed by an 8-month extension phase, obese subjects completing 1 year of treatment with pramlintide 360 Kg twice daily experienced an average weight loss from baseline of 7.9% compared with only 1.1% in subjects receiving placebo.[46] These findings in obese humans are consistent with nonclinical findings and provide validation for the DIO rodent as a relevant model for translational clinical research.

COMBINATION STUDIES WITH AMYLIN + LEPTIN IN RODENT OBESITY: SYNERGISTIC, FAT-SPECIFIC WEIGHT LOSS

The initial studies of amylin administration in otherwise leptin-resistant DIO rats were suggestive of leptin sensitization because weight loss was fat-specific, lean mass-sparing, and not associated with compensatory metabolic adaptations, such as decreased energy expenditure that typically accompany the maintenance of a weight-reduced state.[40]

Amylin restoration of leptin responsiveness was confirmed in a series of nonclinical coadministration studies. First, doses of leptin that had no effect on body weight in leptin-resistant DIO rats clearly amplified amylin-mediated weight loss.[48] Next, amylin and leptin combination treatment was shown to induce greater weight loss and fat loss relative to a control group pair-fed to the amylin-treated group who received leptin treatment, indicating that the greater efficacy observed with combination treatment was not due solely to decreased food intake. Although the combination of leptin with other anorexigenic peptides such as PYY_{3-36} and a glucagon-like peptide-1 analog also resulted in significant weight loss compared with vehicle-treated DIO rats, neither of these combinations achieved as much weight loss as the amylin and leptin combination.[48] In DIO rats pretreated with amylin for 14 days, amylin and leptin combination treatment for 4 weeks led to a 15% vehicle-corrected weight loss versus 8% to 9% for amylin or leptin treatment alone. Finally, comprehensive studies involving response surface methodology, a widely-accepted statistical method for establishing true synergy between 2 drugs,[49] confirmed a significant synergistic interaction between amylin and leptin for reducing food intake and body weight in DIO rats.[50]

Having formally established a marked, reproducible, synergistic interaction between amylin and leptin in otherwise leptin-resistant DIO rats, we next sought to gain insights into the neurobiological basis underlying this phenomenon. Because there is no set of described first-order neurons on which peripherally administered amylin and leptin converge, synergy is more likely attributable to these neurohormones activating complementary neuronal signaling pathways. Several lines of evidence in lean and obese rodent models suggest that the presence of amylin may "prime" the hypothalamus to respond to leptin. For example, amylin knockout mice lacking endogenous amylin have diminished leptin-stimulated phosphorylated signal and transducer activator of transcription 3 (pSTAT3) levels in both the ARC and the VMH.[50] Amylin amplified (by 2-fold) low-dose leptin-stimulated pSTAT3 signaling within the ARC in lean rats.[50] With sustained amylin infusion, ex vivo leptin binding increased within the VMH and the DMH of lean rats.[51] Importantly, amylin augmentation of leptin signaling is also evident in otherwise leptin-resistant DIO rodents. Pretreatment of DIO rats with amylin for 7 days restored leptin-stimulated pSTAT3 signaling within the VMH. The neural substrates of synergy extend beyond the hypothalamus, as amylin pretreatment also augmented basal and leptin-stimulated signaling in the AP.[47] Whereas further neurobiological studies are clearly warranted, collectively these initial observations suggest that the marked weight- and fat-reducing effects of combined amylin/leptin agonism are consistent with the activation of intrinsic synergistic neuronal signaling pathways.

COMBINATION STUDIES WITH PRAMLINTIDE/METRELEPTIN IN HUMAN OBESITY: CLINICAL PROOF-OF-CONCEPT

On the basis of the findings suggesting a synergistic effect of amylin and leptin on weight loss in a relevant rodent model of general obesity, a clinical study[47] involving coadministration of pramlintide and metreleptin for 20 weeks was conducted in overweight and obese subjects with body mass index up to 35 kg/m^2. During a 4-week lead-in phase, subjects

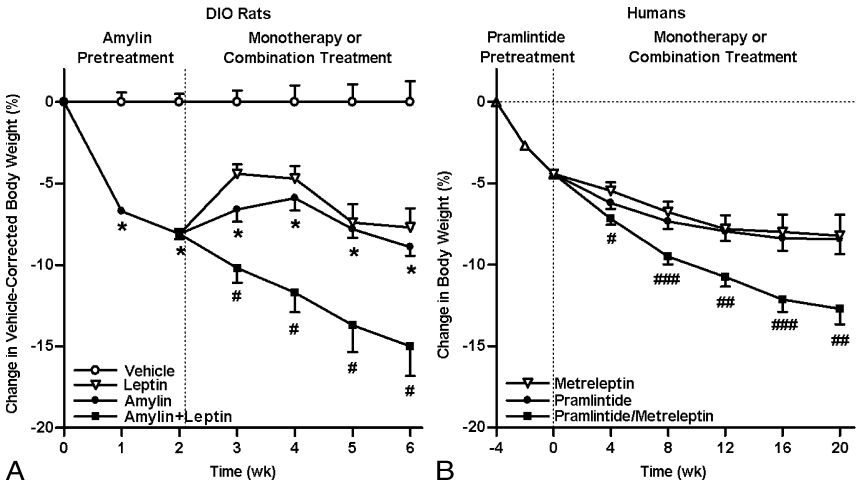

FIGURE 2. Effects of amylin and leptin agonism on body weight in DIO leptin-resistant rats and overweight/obese humans. A, DIO rats were pretreated with amylin (100 μg/kg per day) administered by continuous subcutaneous infusion for 2 weeks and were then treated for 4 weeks with amylin, leptin (500 μg/kg/d), vehicle or amylin + leptin combination treatment. Mean ± SE; $^{*}P < 0.05$ versus vehicle; $^{\#}P < 0.05$ versus monotherapies. B, Change in weight from enrollment (week 4) for subjects (n = 93) pretreated with pramlintide (titrated from 180 to 360 μg twice a day) for 2 weeks and then treated with pramlintide (360 μg twice a day), metreleptin (5 mg twice a day), or pramlintide + metreleptin combination treatment. Evaluable population; least squares mean ± SE; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus monotherapies. Reproduced with permissions.[47]

were treated with pramlintide 180 μg twice daily for 2 weeks, then 360 μg twice daily for 2 weeks along with a 40% calorie-deficit diet (550-1150 kcal/d). Individuals achieving at least 2% to 8% weight loss during the 4-week lead-in were randomized to pramlintide + metreleptin (360 μg and 5 mg twice daily), pramlintide alone (360 μg twice daily), or metreleptin alone (5 mg twice daily) for 20 weeks and instructed to follow a 20% calorie-deficit diet. Of 177 subjects enrolled in the study, 139 were randomized to treatment.

The average weight loss during the 4-week pramlintide lead-in was approximately 4%, which, as expected, was accompanied by an approximately 20% decrease in fasting leptin concentrations. At the end of the 20-week randomized treatment period, subjects receiving pramlintide or metreleptin alone had an average weight loss of approximately 8%, whereas those receiving the combination treatment achieved an average weight loss of 12.7% (Fig. 2). Importantly, unlike the monotherapy groups, the pramlintide + metreleptin treatment group had not reached a weight loss plateau when the study had finished. The combination treatment was generally well tolerated without clinically significant changes in routine safety parameters. The most common adverse effects of coadministration of pramlintide and metreleptin were consistent with that previously observed with either agent administered separately, specifically nausea and injection site reactions, respectively, that were generally mild to moderate and transient.

These findings established a clear proof-of-concept for combined amylin/leptin agonism achieving greater weight loss than either treatment alone. To our knowledge, this is the first clinical study demonstrating that exogenous leptin administration can induce significant, clinically meaningful weight loss in humans with general obesity and, therefore, that metreleptin may have therapeutic utility beyond a strategy of leptin replacement in states of complete or relative leptin deficiency. The therapeutic potential of pramlintide/metreleptin in general obesity is under further investigation in a placebo-controlled dose-ranging study in overweight and obese subjects across a broader body mass index and dose range.

CONCLUSIONS AND OUTLOOK

The discovery of leptin in 1994 opened a new era in obesity research, by demonstrating that body weight is controlled by a complex neurohormonal feedback system. Today, it is widely recognized that multiple adipocyte-, gut-, and islet-derived hormonal signals interact with a complex, distributed network within the CNS to achieve tight control of energy balance and body fat stores. Although multiple nonclinical and clinical studies suggest that leptin itself does not have therapeutic potential as an antiobesity agent, rigorous translational research efforts by academic, government, and industry scientists have yielded important insights into leptin's physiological role and therapeutic potential. The remarkable consistency between nonclinical and clinical findings, including leptin's effects in complete leptin deficiency (ob/ob), low-leptin states (severe lipodystrophy), and general obesity, makes the study of leptin agonism a good example of successful translational research. Nonclinical and clinical studies conducted to date suggest that leptin, a long-term adiposity signal, may have therapeutic potential when used in combination with a short-acting satiety signal such as amylin. Further studies are needed to advance our understanding of the neurobiological basis for amylin/leptin synergy and to confirm whether combined amylin/leptin agonism (with pramlintide/metreleptin) is safe and effective for weight loss, as part of an integrated, neurohormonal approach to obesity pharmacotherapy.

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