Implications of Amylin Receptor Agonism

Integrated Neurohormonal Mechanisms and Therapeutic Applications

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Amylin receptor agonism is emerging as part of an integrated neurohormonal therapeutic approach for managing diabetes mellitus (DM) and body weight. Pramlintide acetate, an analogue of the pancreatic hormone amylin, has been studied in the United States as an antihyperglycemic agent in patients with type 1 or type 2 DM treated with mealtime insulin. Further clinical testing of pramlintide in subjects with obesity demonstrated that pramlintide monotherapy induced significant, sustained, and dose-dependent weight loss. Recent clinical observations point to its compatibility as a combination therapy with the hormone leptin, eliciting double-digit weight loss in patients with overweight and obesity. Herein, we link amylin activation of central neural circuits to these therapeutic effects, and we speculate on other potential therapeutic applications of amylin receptor agonism.

PHYSIOLOGIC EFFECTS OF AMYLIN

Amylin is a 37–amino acid peptide hormone cosecreted with insulin from pancreatic β cells. In rodents and humans, plasma amylin concentrations rapidly rise several-fold in response to meals, with a diurnal profile that is almost superimposable on that of insulin. The physiologic effects of amylin receptor agonism include (1) decreased food intake, (2) slowing of the rate of gastric emptying, and (3) reduction of postprandial glucagon release in a glucose-dependent manner. Through these mechanisms, amylin receptor agonism reduces the rate of glucose entry into the bloodstream after meals to better match the ability of insulin to dispose of blood glucose.

AMYLIN BINDING SITES AND NEURONAL ACTIVATION

On secretion into the circulation, amylin binds with high affinity to receptors in the central nervous system. Within the brain, amylin-specific receptors, which are composed of the calcitonin receptor partnered with individual receptor-modifying proteins, are located in the nucleus accumbens, the dorsal raphe, and the hindbrain area postrema. There is evidence of specific binding of amylin to receptors located in the nucleus accumbens and dorsal raphe, but, because these areas reside within the blood-brain barrier, it is unlikely that their cognate ligand is circulating amylin. The role of these binding sites remains to be elucidated.

THE AMYLIN NEURAL CIRCUIT

Area Postrema

The area postrema (Figure), which lacks a blood-brain barrier, has a pivotal role in mediating the physiologic effects of amylin by receiving and integrating peripheral meal-related signals. Approximately 90% of neurons in the area postrema that express amylin-specific receptors also express glucose-sensing receptors. In a rat model, lesions in the area postrema reduced the anorexigenic and gastric emptying effects of peripherally administered amylin, identifying the area postrema as an essential location for amy-
Similarly, blockade of endogenous amylin in the area postrema via central administration of the selective amylin antagonist AC187 increased food intake and reversed the anorexigenic effects of peripherally administered amylin. The amylin antagonist AC187 also raised glucagon concentrations, accelerated gastric emptying of liquids, and increased glycemia after an oral nutrient challenge, consistent with a central role of amylin in regulating nutrient intake and use.

**Nucleus of the Solitary Tract, Lateral Parabrachial Nucleus, and Central Amygdala**

Whereas the hindbrain area postrema clearly contains the “first-order” set of neurons that transduce the effects of peripheral amylin, amylin affects other areas of the brain via well-established neural pathways. Major reciprocal projections exist between the area postrema, the adjacent area of the solitary tract, and the lateral parabrachial nucleus (Figure). In turn, the lateral parabrachial nucleus projects to the central nucleus of the amygdala. These nuclei seem to be sequentially stimulated (as measured by c-fos, a marker of neuronal activation) in response to amylin receptor stimulation in the area postrema, collectively mediating the anorexigenic effects of amylin. Lesions of the area postrema abolish neuronal activation in the lateral parabrachial nucleus and the central nucleus of the amygdala, whereas lesions of the lateral parabrachial nucleus abrogate signaling in the central nucleus of the amygdala following amylin administration.

**Hypothalamus**

The importance of amylin in modulating hypothalamic responses to nutritional status and other weight regulatory signals has been established in several nonclinical studies in lean or diet-induced obese rodents. In short-term investigations in lean animals, peripheral amylin injection reversed fasting-induced c-fos expression in the lateral hypothalamic area, mirroring the effects of refeeding, and downregulated the expression of lateral hypothalamic orexigenic genes. These effects are likely indirect because amylin binding has not been reported in this nucleus. The anorexigenic effects of amylin may also include cross-talk with hypothalamic histaminergic signaling pathways because histaminergic receptor blockade within the ventromedial hypothalamus blunted the anorexigenic effects of amylin. Finally, the short-term satiating effect of peripheral amylin was enhanced by central administration of the adiposity signals leptin or insulin, which act in the hypothalamus to reduce feeding. In diet-induced obese leptin-resistant rats, the effects of peripheral amylin treatment were consistent with enhanced central responsiveness to leptin. Amylin-mediated weight loss (but not caloric restriction) was associated with increased pro-opiomelanocortin gene expression (a downstream target of leptin and the precursor of the anorectic α-msh) within the arcuate nucleus. In addition, whereas vehicle-treated diet-induced obese rats have diminished hypothalamic leptin signaling, pretreatment with amylin (but not caloric restriction) for 1 week restored leptin-induced neuronal activation (eg, pSTAT-3 [phosphorylated STAT3] signaling) within the ventromedial hypothalamus.

**Other Regions of the Brain**

It is possible that pharmacologic levels of amylin engage additional neural circuits. Rich amylin binding has been demonstrated in the nucleus accumbens, a key brain region mediating food reward, suggesting that amylin signaling may influence hedonic responses to food. Consistent with this hypothesis, food choice experiments showed that intake of highly palatable (high-fat or high-sucrose) foods was selectively decreased during short- and long-term amylin treatments. However, it is unlikely that endogenous circulating levels of amylin cross the blood-brain barrier to directly activate this nucleus. The mechanism of the role of amylin in food reward remains to be explored.

In summary, these preclinical findings demonstrate a role for amylin receptor agonism in regulating blood glucose concentrations and in integrating feeding-, gut-, and taste-
related signaling. Amylin transmits the integrated information to upstream nuclei involved in energy balance and possibly mediates food reward.

CURRENT AND POTENTIAL THERAPEUTIC APPLICATIONS

The ability of amylin to stimulate key neural circuits involved in blood glucose regulation and food intake led to the investigation of amylin analogues as treatments for metabolic diseases. Herein, we highlight how amylin-dependent activation of neural circuits can be leveraged in the treatment of DM and potentially in obesity and neuropsychiatric diseases.

Diabetes Mellitus

Diabetes mellitus is characterized by hyperglycemia resulting from defects in the secretion or action of multiple hormones, including insulin, amylin, glucagon, and glucagon-like peptide 1. Dysfunction of pancreatic β cells is a core defect in DM that results in reduced (type 2 DM) or absent (type 1 DM) secretion of insulin and amylin in response to food intake. Insulin therapy is a common treatment for patients with DM. However, many patients who use insulin are unable to maintain adequate glycemic control perhaps in part because of the continued dysregulation of other hormones such as amylin.

Pramlintide, an analogue of human amylin, was developed to overcome several physicochemical properties that make human amylin unsuitable for pharmacologic delivery. By mimicking the aforementioned neurohormonal actions of amylin, pramlintide complements the actions of insulin by regulating the presence of glucose in the circulation via slowed gastric emptying, reduced food intake, and decreased postprandial glucagon secretion. In clinical investigations of patients with type 1 or type 2 DM, pramlintide reduced glycated hemoglobin and postprandial glucose excursions with a concomitant reduction in insulin dosage, improving overall glycemic control compared with insulin treatment alone. In addition, pramlintide treatment generally led to weight loss, while insulin treatment tended to promote weight gain. The most frequent treatment-emergent adverse events with pramlintide use were mild to moderate insulin-induced hypoglycemia and nausea, which decreased over time. Collectively, findings from these studies suggest that pramlintide in combination with insulin provides a physiologic approach to the treatment of DM by mimicking the effects of amylin.

Obesity

Results of preclinical investigations suggest that amylin may be useful for treating obesity. Amylin administered peripherally to rodents decreased meal size and increased the ratio of postmeal interval to meal size (a measure of satiety in humans) without any indication of malaise (kaolin intake, locomotor activity, or taste aversion). Reductions in food intake and body weight have been maintained for up to 11 weeks of treatment with continuous peripheral amylin administration in rat models of diet-induced obesity. The weight-reducing effects of amylin are dose dependent, are not associated with compensatory decreases in energy expenditure, and are attributed to the loss of fat mass with relative preservation of lean mass. Whereas reduced food intake is the predominant mechanism of overall weight loss, pair-feeding investigations demonstrate that amylin-treated rats lose more fat than would be predicted due to caloric restriction alone.

These preclinical findings with amylin, along with the observation that pramlintide therapy reduced body weight in patients with insulin-treated DM, pointed to its potential clinical usefulness as an antoobesity agent. In subjects with obesity, pramlintide elicited significant weight loss in the absence of lifestyle intervention. When used in conjunction with lifestyle intervention, pramlintide use led to greater initial weight loss compared with placebo (5.7% vs 2.6% at 4 months) and longer maintenance of weight loss (7.9% vs 1.1% at 12 months). The most common adverse event in these studies was mild to moderate nausea, which decreased over time.

Monotherapies that target a single aspect of the multihormonal dysregulation associated with obesity may be limited in efficacy by compensatory mechanisms that favor weight maintenance and regain. For example, weight loss and energy restriction (as encountered during dieting or with drug treatment) trigger a cascade of events to defend body weight, including a fall in leptin level (due to fat loss), decreased sympathetic nervous system activity, increased muscle work efficiency, reduced energy expenditure and metabolic rate, increased hunger, and decreased satiety and fullness. There is a growing consensus that overcoming these compensatory mechanisms will require combination therapies that target multiple mechanisms regulating body weight. Our translational obesity research program has recently explored the following 2 combinatorial strategies: (1) amylin-leptin agonism to harness short-term satiety and long-term adiposity signaling and (2) amylin receptor agonism combined with approved small-molecule anorectics that act via classic neurotransmitter systems.

Preclinical studies demonstrated synergistic weight and fat loss when amylin was coadministered with leptin in rats with diet-induced obesity and additive weight loss when amylin was coadministered with the small-molecule anorectics sibutramine hydrochloride or phentermine hydrochloride. In a randomized double-blind clinical proof-of-concept study in subjects with overweight and obesity, coadministration of metreleptin and pramlintide acetate for 20 weeks (after a 4-week lead-in treatment period with pramlintide alone) elicited significantly more weight loss (approximately 13%) than either treatment alone. In a randomized 24-week single-blind study (only the subjects were blinded to study medication), the combination of pramlintide with sibutramine or phentermine significantly increased weight loss (approximately 11 kg) compared with pramlintide monotherapy (approximately 4 kg). The safety pro-
files in both of these studies were consistent with those of the individual treatments, suggesting that targeting multiple pathways through the combination of an amylin agonist and centrally acting anorectic agents may lead to enhanced weight loss without the emergence of unexpected adverse events. Together, the findings of these studies suggest that a combinatorial approach that includes pramlintide may successfully override the central nervous system mechanisms for defending body weight.

Neuropsychiatric Disease

Amylin activation of central neural circuits may also have therapeutic potential in the treatment of neuropsychiatric diseases. It is increasingly clear that the neural circuitry modulating energy homeostasis interacts and even overlaps with circuits involved in cognition, reward, and stress. Hence, the peptides and proteins propagating these signals may have beneficial emotional or behavioral effects, as well as effects on metabolic (or energy-related) processes.

In preclinical studies, amylin receptor agonism has recently been associated with anxiolytic and antidepressant properties. Long-term stress triggers a dietary imbalance that increases palatable feeding and visceral obesity through resetting of the hypothalamic-pituitary-adrenal axis. When standard chow and sucrose were made freely available to rats, amylin administration not only decreased preference for sucrose but also prevented stress-induced sucrose consumption following restraint stress. In models of depression and anxiety, amylin decreased immobility in the forced swim test, reduced marble burying, increased the number of crossings in the four-plate test, and attenuated the hyperthermic response to restraint stress, which was blocked following lesioning of the area postrema. Furthermore, stress-induced c-fos activation in central circuits was reduced with amylin treatment. Although these intriguing preclinical observations require clinical validation, they suggest that the integrated mechanisms of amylin may improve metabolic and behavioral processes.

CONCLUSIONS

The direct and indirect effects of amylin receptor agonism are summarized in the Table. In addition to its known therapeutic effects on glycemic control in patients with type 1 or type 2 DM using insulin, amylin receptor agonism is emerging as a novel potential therapy for obesity as monotherapy or in combination therapy. It is increasingly clear that activation of the amylin neural circuit may have usefulness beyond the treatment of metabolic diseases. Nonclinical evidence suggests therapeutic potential of amylin in neuropsychiatric diseases. Further research on amylin receptor agonism in several other therapeutic areas is clearly warranted. Recent efforts to develop additional amylin analogues have indicated that it is possible to enhance specific amylin actions. For example, the amylin analogue AC2307 was recently shown to be more potent and to elicit greater weight loss relative to native amylin in nonclinical investigations. To what extent specific amylin actions can be individually optimized to treat various disease states remains to be determined.

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