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Amylin-mediated control of glycemia, energy balance, and cognition

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Abstract

Amylin, a peptide hormone produced in the pancreas and in the brain, has well-established physiological roles in glycemic regulation and energy balance control. It improves postprandial blood glucose levels by suppressing gastric emptying and glucagon secretion; these beneficial effects have led to the FDA-approved use of the amylin analog pramlintide in the treatment of diabetes mellitus. Amylin also acts centrally as a satiation signal, reducing food intake and body weight. The ability of amylin to promote negative energy balance, along with its unique capacity to cooperatively facilitate or enhance the intake- and body weight-suppressive effects of other neuroendocrine signals like leptin, have made amylin a leading target for the development of novel pharmacotherapies for the treatment of obesity. In addition to these more widely studied effects, a growing body of literature suggests that amylin may play a role in processes related to cognition, including the neurodegeneration and cognitive deficits associated with Alzheimer's disease (AD). Although the function of amylin in AD is still unclear, intriguing recent reports indicate that amylin may improve cognitive ability and reduce hallmarks of neurodegeneration in the brain. The frequent comorbidity of diabetes mellitus and obesity, as well as the increased risk for and occurrence of AD associated with these metabolic diseases, suggests that amylin-based pharmaceutical strategies may provide multiple therapeutic benefits. This review will discuss the known effects of amylin on glycemic regulation, energy balance control, and cognitive/motivational processes. Particular focus will be devoted to the current and/or potential future clinical use of amylin pharmacotherapies for the treatment of diseases in each of these realms.

Keywords

IAPP; obesity; pramlintide; Alzheimer's; leptin; motivation

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1. Introduction

One commonly observed feature in the pancreas of individuals with type 2 diabetes mellitus (T2DM) is the presence of insoluble amyloid aggregations, or deposits of protein folded in β -sheets, in the islets of Langerhans. Although the precise mechanisms by which these deposits develop are still not entirely clear, initial identification of amyloid in the pancreas of diabetic patients dates back to the early 1900s [1]. Later reports confirm the presence of pancreatic amyloid deposits in the islets of humans [2, 3], non-human primates [4, 5], and cats [6, 7] with diabetes. Characterizing the composition of the amyloid aggregations took decades, but eventually it was determined that the amyloid comprised a previously unknown 37-amino acid peptide, which was termed islet amyloid polypeptide (IAPP) [8, 9] or, less commonly, diabetes-associated peptide [10].

Intriguingly, although aggregated IAPP is a prominent feature in pancreatic tissue from diabetic individuals, IAPP was also found in the pancreatic islets of non-diabetics [9]. This suggested that IAPP might have an important role in the normal endocrine function of the pancreas. Later research provided evidence that IAPP itself is not inherently cytotoxic, supporting the notion that it may have physiologically relevant effects under normal conditions. Rather, the process of IAPP aggregation disrupts normal cellular function and can induce cell death [11, 12]. As researchers began to uncover physiological effects of IAPP in non-disease states, thus demonstrating roles for the peptide beyond its association with pathophysiological amyloid deposits, it was renamed amylin [13-15], which remains the most commonly used name today.

Indeed, we now recognize that amylin has important neuroendocrine functions in glycemic regulation (see [16-18] for review). Amylin is co-secreted with insulin from the pancreatic β -cells in response to nutrient stimuli during a meal, and suppresses postprandial blood glucose levels [18-23]. The beneficial glycemic effects of amylin led to the development and subsequent clinical usage of an FDA-approved amylin analog, pramlintide, as an adjunctive therapy for the treatment of diabetes mellitus [24, 25]. However, amylin also has potent effects on energy balance, and acts within the brain to reduce food intake and body weight [26, 27]. It is important to note that the energy balance and glycemic effects of amylin are dissociable under carefully controlled experimental conditions [28]. Collectively, these metabolic effects of amylin have brought attention to this peptide as a leading candidate for the development of new pharmacotherapies for the treatment of obesity and diabetes [27, 29-32].

Recent research has brought to light a putative novel role for amylin in processes related to cognition, including possible ameliorative effects on neurodegenerative and psychotic disorders [33, 34]. Although this area of research is still relatively new, the notion that amylin-based pharmacotherapies could be used for treatment of such disorders is already being considered [35]. Here, an overview of amylin's roles in glycemic regulation, energy balance control, and cognitive processes will be provided. In addition, the current and potential future clinical use of pharmaceuticals targeting the amylin system to treat disorders related to each of these realms will be discussed.

2. Glycemic regulation

Normal glycemic regulation involves coordinated physiological effects that maintain adequate levels of available glucose; the specific responses required depend on the energy status of the individual. Simply put, when a human or non-human animal is fasted, glycemic regulation primarily entails the conversion of stored energy to readily usable energy (e.g., conversion of liver glycogen to glucose), a process initiated by the pancreatic hormone glucagon. When a non-human animal or human is actively consuming or has recently consumed food, a different set of responses is required to control the influx of glucose from the food while suppressing the endogenous production of glucose from glycogen. Amylin is released during the feeding/fed state in response to nutrient entry into the gastrointestinal tract. Accordingly, amylin and amylin receptor agonists reduce postprandial blood glucose levels and improve glycemic control in humans and in non-human animal models [18]. The mechanisms by which this occurs, and the current use of amylin-based drugs for the treatment of diabetes mellitus, will be discussed in this section.

2.1 Amylin-mediated control of blood glucose levels in normoglycemic individuals

When a human or other animal eats, amylin is released with insulin from pancreatic β -cells [18-23]. The roles of amylin and insulin for glycemic regulation have been suggested to be complementary [16, 18], rather than a direct functional interaction. The effects of insulin on blood glucose levels are well known; this hormone facilitates transport of glucose from the bloodstream into peripheral tissues such as skeletal muscle (recently reviewed in [36]). However, blood glucose levels are influenced not only by the transport of glucose into tissue, but also the entrance of glucose from ingested food into the bloodstream. This side of the equation is controlled in part by amylin, which slows gastric emptying [37, 38], thereby delaying and controlling the entry of nutrients/glucose into the small intestine and subsequently into circulation. This effect can be observed when plasma amylin is experimentally increased to levels comparable to those normally observed in a postprandial state [39].

The available evidence indicates that amylin may act centrally to inhibit gastric emptying. In one study [40], subdiaphragmatic deafferentation was carried out in rats according to the procedure of Walls, Powley, and colleagues [41], which eliminates vagal afferent signaling while maintaining a portion of vagal efferent communication. This manipulation did not impact the ability of amylin to suppress gastric emptying in the animals [40], suggesting that the effect may be mediated by direct action in the brain. The area postrema (AP), a nucleus in the caudal hindbrain that is important for the physiological and behavioral effects of amylin [30, 42], may be one important central site of action for the effects of amylin on gastric emptying. Lesions of the AP appear to diminish the ability of amylin to suppress gastric emptying (reviewed in [43]). However, the neural mechanisms by which amylin might act in the AP to impact gastric emptying are unclear. The dorsal vagal complex of the hindbrain, comprising the AP, the nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus (DMV), plays a critical role in the neural control of gastric emptying (reviewed in [44, 45]). Briefly, the NTS integrates relevant neural and humoral signals, including direct vagal input, and sends this information to the DMV. Gastric-projecting

preganglionic neurons in the DMV then provide autonomic control over gastric emptying (reviewed in [45]). It is clear that the AP projects to the NTS [46] and that peripheral amylin induces cFos in both of these nuclei [47]. To speculate, amylin may act in the AP, or even within the NTS itself [48], to provide excitatory input to NTS neurons [47, 49], perhaps even by complementing glutamatergic vagal input to the NTS [50-52]. Excitation of the NTS can alter the activity of DMV neurons [53], and if preganglionic neurons that control gastric emptying in the stomach are impacted, this could provide a neural mechanism by which AP/NTS amylin suppresses gastric emptying. Yet, our understanding of the ability of amylin to directly or indirectly modulate the activity of dorsal vagal complex nuclei for the control of gastric emptying is currently quite limited, and clearly the ability of amylin to activate this potential pathway must be tested.

Furthermore, the possibility that amylin may act directly on the stomach to suppress gastric emptying should not be excluded. Although the primary peripheral site of amylin production is the pancreas, amylin mRNA is expressed in several other sites in rodents, including the hypothalamus [54], dorsal root ganglia [55, 56], lung [55], and gastrointestinal tract [55-57]. Indeed, amylin mRNA is expressed in the stomach and pylorus of both humans and rats [55, 58], and amylin binding is observed in the stomach fundus in rats [59]. However, the ability of amylin to impact stomach muscle relaxation has not been widely studied or reported. One paper indicates that amylin exerted a relaxant effect on isolated strips of rat ileal smooth muscle [60], a direction of effect consistent with inhibition of gastric motility and gastric emptying. Whether this effect also applies to rat stomach muscle, or indeed whether similar effects occur *in vivo*, are open questions. If direct actions of amylin on stomach musculature are detected, an important follow-up question will be whether the source of that amylin is pancreatic or extra-pancreatic. Amylin mRNA levels in the gastrointestinal tract are far lower than those in the pancreas [55, 57], but nevertheless, radioimmunoassay detection of gastrointestinal amylin peptide has been reported in rats [61]. The potential contributions of alternative, extra-pancreatic sources of amylin to the physiological and behavioral effects of the peptide, including effects on gastric emptying, need to be addressed empirically.

In addition to amylin's role in the normal postprandial state, it is critical to consider the effects of amylin on gastric emptying in situations where a delay in emptying would be maladaptive, such as when blood glucose levels are low. During hypoglycemia, gastric emptying is accelerated to increase available glucose as rapidly as possible [62, 63], and concomitantly, amylin's suppressive action on this process is abolished [64]. The neural mechanisms underlying this "hypoglycemic override" of amylin's gastric emptying effects are not completely understood. However, it may be mediated in part by neurons in the AP that respond to both amylin and glucose [65]. Specifically, within the AP, the majority of neurons that are activated by amylin [66] are inhibited by low concentrations of glucose [65], suggesting that prevailing hypoglycemia may block or at least diminish the capacity of these neurons to respond to amylin. The fact that destruction of the AP blocks the gastric emptying effects of amylin [43] hints at the possibility that this amylin- and glucose-responsive neuronal population may be involved in these effects, but this idea needs to be evaluated empirically.

Another important aspect of glucose regulation during the fed state is to prevent continued endogenous production of glucose from energy stores when ingested glucose is high. Amylin also is involved in this aspect of glycemic control; it suppresses glucagon release from the α -cells of the pancreas [67], thus inhibiting the actions of glucagon to promote hepatic gluconeogenesis and glycogenolysis (for review, see [68]).

2.2 Dysregulation of amylin in diabetes mellitus and pharmacological treatment with pramlintide

In healthy humans, postprandial release of amylin generates plasma concentrations in the picomolar range [69, 70], concentrations sufficient to exert glycemic effects. However, amylin becomes dysregulated in individuals with diabetes mellitus, a disease characterized by long-term hyperglycemia due to a loss of or impairment in insulin signaling. Insufficiency of insulin signaling may be due to autoimmune destruction of the pancreatic β -cells themselves (type 1 diabetes mellitus, or T1DM) or to the target cells of insulin failing to respond to the hormone and, eventually, consequent reduction in insulin production as the β -cells become disrupted (T2DM; see [71] for a recent review of these subtypes). The presence of pancreatic amyloid is observed in both subtypes, although whether amyloid deposits are a cause or a consequence of diabetes mellitus remains unresolved [72, 73].

The fact that amylin levels are also disrupted in the diabetic state is logical given that its release is highly correlated with that of insulin under most conditions [74-77]. In T1DM, levels of amylin are extremely low in the plasma and in the pancreas itself [69, 78-81], as one would expect in a condition where the pancreatic source of amylin is destroyed. In contrast, amylin levels in T2DM depend on the progression of the disease. In earlier stages of T2DM, hyperamylinemia is observed [82]; however, as the disease progresses and the β -cells fail, amylin levels fall [83].

In both T1DM and more advanced cases of T2DM, amylin levels are low; this led to the notion that replacing amylin might be therapeutically useful for the treatment of diabetes [84]. In particular, it was thought that restoring amylin signaling could be beneficial for slowing the abnormally rapid gastric emptying rate of individuals with diabetes [85]. This pathophysiological effect can exacerbate postprandial hyperglycemia, as nutrients enter the small intestine and are absorbed more rapidly than normal; replacing amylin could potentially reverse this symptom and thereby normalize glycemia [84, 86, 87]. However, human amylin is highly amyloidogenic, or prone to pathological aggregation, due to the presence of amino acid sequences that are disposed to β -sheet fibril formation [88, 89]. Therefore, development of an amylin agonist that would avoid this property while still producing the beneficial glycemic effects of amylin was required, and thus the non-amyloidogenic amylin analog pramlintide was developed [90].

Today, pramlintide is FDA-approved for the treatment of both T1DM and T2DM [91]. It is used as an adjunct therapy along with exogenous replacement of insulin. It is administered subcutaneously before meals, but must be injected separately from insulin due to pH/solubility concerns [92]. Pramlintide recapitulates the glycemic effects of native amylin; it effectively suppresses postprandial blood glucose levels [86, 93], reduces the rate of gastric emptying [94-97], and inhibits glucagon [98, 99] in humans. The most commonly reported

adverse effect of pramlintide is nausea [100-104]. When pramlintide is used in combination with insulin, there is also a chance of inducing severe hypoglycemia [105], so doses of each drug need to be titrated carefully to mitigate this risk.

Although pramlintide has been used clinically for about a decade, there is always an interest in improving what is already available, or even developing novel amylin-based strategies for the pharmacotherapeutic management of diabetes. As mentioned briefly, one of the downsides of pramlintide is that it must be injected separately from insulin. The rationale for this is that pramlintide is soluble in a more acidic solution (pH ~4) [106] whereas injectable insulin is formulated at a more neutral pH in the range of ~7-8 [92]. Therefore, one area of research involves identifying new amylin analogs that are more soluble at neutral pH levels, yet are non-amyloidogenic [107]. Another strategy currently being explored is to reduce degradation of endogenous amylin, thereby prolonging its beneficial effects. Renal clearance is largely responsible for the breakdown of amylin and analogs like pramlintide [108]. This may occur at least in part by the actions of insulin-degrading enzyme (IDE) [109], which is highly expressed in the kidney [110]. Blocking IDE activity in mice increased plasma amylin concentrations [111] leading some to conclude that this may be a viable strategy for treating T2DM [111, 112], perhaps by combining an anti-IDE pharmacotherapy with an existing drug like pramlintide. These areas of research are quite new, and clearly, many questions remain to be answered regarding the possible translational relevance of these strategies.

3. Energy balance control

3.1 Central sites of action for amylin in the control of food intake and body weight

In addition to its potent glycemic effects, amylin has an important role in the control of energy balance. Specifically, amylin reduces food intake and body weight, and acts as a satiation signal to promote negative energy balance [26, 113, 114]. Peripheral or central administration of amylin or an amylin receptor agonist can induce hypophagia and weight loss [113-118]; these effects are mediated via direct action in the central nervous system [119]. Amylin also influences energy expenditure [120, 121], although this has not been as extensively investigated. This section will focus on the feeding and body weight effects of amylin, which have been more widely studied.

Research on the sites of action within the brain for amylin's intake- and body weight-suppressive effects has focused primarily on the AP of the hindbrain. A large body of literature indicates that amylin receptor activation in the AP suppresses food intake [122-124]. Furthermore, reducing the ability of amylin to act at the AP via lesioning of this nucleus [42, 125] or by pharmacological blockade of amylin receptors [122] attenuates the anorectic effects of peripherally-administered amylin. The importance of the AP for amylin's effects on energy balance has been a focus of several comprehensive reviews [30, 126, 127].

Although the AP is certainly an important and physiologically relevant site of action for amylin's energy balance effects, amylin binding sites are found not only in the AP, but in fact are distributed widely throughout the brain [128-130], including areas known to be important for the control of feeding and motivated behavior such as hypothalamic nuclei,

structures of the mesolimbic system, and other feeding-relevant nuclei in the caudal brainstem. Coupled with our ever-growing appreciation of the distributed nature of energy balance control [131, 132], the existence of amylin binding sites throughout the neuraxis suggests that at least some of these sites likely contribute directly to the control of energy balance by amylin. Further support for this notion comes from the fact that amylin can cross the blood-brain barrier [133, 134], presenting the intriguing hypothesis that sufficient concentrations of circulating amylin may be able to directly access numerous parenchymal targets to influence energy balance.

The hypothesis that amylin directly acts in a variety of nuclei to control food intake and body weight has begun to receive more attention in the last few years. This is an especially important empirical research theme given the recognition that the amylin system is a promising target for the development of pharmacotherapies to treat obesity [29, 31, 32]. Our laboratory has focused on the role of the ventral tegmental area (VTA), a major nucleus of the mesolimbic reward system, in mediating the energy balance effects of amylin receptor activation. The VTA is an important site for motivated behavior, including the control of food intake [135-137], and several peripherally-derived feeding-related peptides act directly in the VTA to alter food intake and motivation for food [138-142]. Our work has demonstrated that amylin receptor signaling in the VTA is both pharmacologically and physiologically relevant for the control of food intake. Activation of amylin receptors in the VTA with the amylin receptor agonist salmon calcitonin (sCT) [143, 144] or amylin itself [145] suppresses food intake in rats. Furthermore, reducing VTA amylin receptor signaling increases feeding. This effect was observed in an acute experiment using a pharmacological amylin receptor antagonist, AC187 [143], as well in a chronic study availing of a novel adeno-associated virus to knock down amylin receptor expression in the nucleus [144], supporting the relevance of the VTA for amylin-mediated control of energy balance over the short-term (~24h) and long-term (~1 month). The anorectic and body weight-suppressive effects of VTA amylin signaling appear to be mediated, at least in part, by suppression of dopaminergic signaling from the VTA to the nucleus accumbens core (NAcC), one of the major afferent targets of VTA dopamine neurons and a site that itself is important for mediating responses to internally- and externally-derived signals related to feeding [144]. Interestingly, VTA amylin receptor signaling may have more powerful suppressive effects on the intake of palatable foods than of a bland food, at least over the longer-term [144].

The nucleus accumbens (NAc) represents another major nucleus of the mesolimbic reward system and in fact was one of the first nuclei in the brain recognized for its robust binding of amylin [128]. However, the role of direct amylin signaling within the accumbens for the control of food intake is still unclear. One report suggests that injection of amylin in the nucleus accumbens shell (NAcSh), but not NAcC, subregion reduces food intake [146]. However, when an alternate, angled cannula placement was used to target the shell, thus avoiding potential ventricular diffusion of the injection, food intake was unchanged [146]. A more recent microinjection study in the NAcSh, in which the ventricles were avoided, suggests that amylin may act in the NAcSh to suppress mu-opioid-induced hyperphagia [147]. This paper also demonstrates that direct intra-NAcSh infusion of amylin reduces sucrose intake, as well as food deprivation-induced chow intake, albeit at higher doses of amylin than those required to modify opioidergic feeding [147]. These collective findings

suggest that amylin in the NAcSh may impact food intake primarily by interacting with and modulating other feeding-relevant signals, in contrast to its ability to independently influence feeding via actions at other nuclei like the AP and the VTA.

3.2 Amylin production in the brain

When considering the energy balance effects of amylin in targets such as the VTA and NAc, which are protected by the blood-brain barrier, the endogenous source of amylin to these nuclei becomes an important question. The primary known source of amylin in the body is the pancreas, and although amylin can cross the blood-brain barrier [133, 134], does circulating, pancreatic-derived amylin actually access these sites to cause hypophagia and weight loss? To date, studies have only indirectly addressed this question. For example, in the VTA, acute blockade of amylin receptors with the antagonist AC187 increases food intake [143], but the source of the endogenous amylin is unknown and can only be assumed to be the pancreatic β -cells. However, exciting new research suggests that amylin may also be produced in the brain.

The first evidence of central amylin production came from studies of maternal female rats, and demonstrated expression of amylin mRNA in the preoptic area, medial preoptic nucleus, and bed nucleus of the stria terminalis in maternally-behaving female rats (post-parturition or sensitized virgin females) [148, 149]. No significant levels of amylin mRNA were observed in these brain areas in male rats or in non-maternal females [149], which led the authors to conclude that at least in these brain areas, amylin was likely exerting effects on maternal behavior specifically. It is important to note, however, that these papers examined amylin expression in fairly restricted areas of the brain, and did not test expression throughout the entire neuraxis.

More recently, Friedman's group examined hypothalamic expression of amylin in mice, within the context of feeding and energy balance. Their results indicate that amylin is expressed in numerous areas of the hypothalamus, including the preoptic area, as well as feeding-related nuclei such as the lateral hypothalamus (LH) and the arcuate nucleus [54]. Importantly, expression was observed in both female and male mice, although expression was higher in females [54]. These novel findings demonstrate the existence of a central source of amylin in both sexes, and raise many new empirical questions as to the projection targets and functions of this centrally-produced amylin. An earlier report shows that neurons in the lateral parabrachial nucleus (LPBN) that are activated by peripheral administration of amylin project to the LH [150], highlighting the possibility that amylin-expressing LH neurons may be part of a broader amylin-responsive neural network that is activated by peripheral amylin. To speculate, although these LPBN neurons inhibit neurons in the LH [150], the phenotype of these LH neurons is unknown. These could be GABAergic interneurons [151], and LPBN inactivation of this population thus might represent removal of inhibition on aminergic LH neurons, thereby increasing LH amylin signaling and providing a central "boost" to the peripherally-derived amylin. This is purely speculative but represents an interesting possibility introduced by this new finding.

3.3 Pramlintide pharmacotherapy in humans promotes negative energy balance

Amylin and amylin receptor agonists suppress food intake and body weight not only in rodent models, but also in humans. For example, acute subcutaneous injection of the amylin analog pramlintide suppressed energy intake in normal-weight males [152]. Importantly, pramlintide is also effective to reduce feeding in obese men [153]. Other feeding-related signals such as the adipose-derived hormone leptin have diminished ability to promote negative energy balance in the obese state [154, 155]; the fact that amylin receptor agonists remain effective to suppress feeding and body weight in the obese state [153, 156-158] is a feature that makes the amylin system an attractive candidate for the development of new obesity treatments.

The use of pramlintide, or any amylin analog, to treat obesity in humans would require chronic administration; accordingly, several studies have focused on the energy balance effects of longer-term pramlintide treatment in obese humans [157, 159-161]. The results consistently demonstrate pramlintide-mediated reductions in energy intake and body weight in obese individuals with or without a concurrent diagnosis of diabetes mellitus [102, 157, 159, 162]. Using pramlintide in combination with behavioral modification may be a beneficial strategy for weight loss [160]. One study suggested that pramlintide may also provide benefits for binge eating, as chronic administration of the drug lowered self-reported binge eating scores [157]. Like glycemic studies, the most common side effect of pramlintide reported in these experiments was nausea, although this is often described as “mild” (e.g., [159, 160]). However, nausea may not be a primary explanation for weight loss, because weight loss was similar in pramlintide-treated individuals who did or did not experience nausea [159]. These data underscore the putative utility of pramlintide or other amylin-based compounds as anti-obesity pharmacotherapies, but it is critical to note that pramlintide is not currently FDA-approved for the treatment of obesity.

3.4 Amylin and leptin cooperatively interact to suppress food intake and body weight

Another feature that makes amylin an attractive candidate for anti-obesity pharmaceutical development is its ability to interact with and enhance the effects of other feeding-related neurohormonal signals. An enhanced suppression of food intake has been observed when amylin is given in combination with other feeding-related peptides such as cholecystokinin [122], peptide YY [163], and insulin [164]. Perhaps most well-studied to date is the ability of amylin to interact cooperatively with the adipose hormone leptin [165-171]. When leptin and amylin are administered together, the suppression of food intake and body weight that is produced by the combination of the two peptides is greater than would be expected based on the effects of each peptide in isolation (i.e., a greater-than-additive effect) [168, 169]. The reduction in body weight involves a selective reduction in adiposity with conservation of lean mass [165, 166, 171]. The neural mechanisms underlying this effect are still being elucidated, but several studies suggest that amylin enhances the effects of leptin. This occurs even during a state of obesity-induced leptin insensitivity or “resistance”, where amylin is able to restore sensitivity to leptin [165, 166, 169].

The neural substrates underlying the cooperative ability of amylin and leptin to enhance suppression of food intake, body weight, and adiposity are still being determined. The

ventromedial nucleus of the hypothalamus (VMN) appears to be one site of interaction. Peripheral administration of amylin enhances leptin binding in this area of the brain of normal-weight animals [169]. In cell line studies, amylin and leptin engage several shared intracellular signaling pathways, including STAT3, Akt, and ERK [172-174]. To date, research has supported the importance of the STAT3 pathway in the interaction of amylin and leptin. In diet-induced obese rats with reduced sensitivity to leptin, peripheral amylin treatment restored leptin-mediated induction of phosphorylated STAT3 (pSTAT3) in the VMN [165]. Furthermore, amylin knockout mice treated with leptin have a blunted pSTAT3 response in this nucleus [169]. The ability of amylin to enhance leptin signaling in the VMN relies on amylin-mediated induction of interleukin-6 (IL-6); blocking IL-6 signaling reduced the ability of amylin to enhance leptin-induced pSTAT3 in this nucleus [175].

The VTA is also a site of interaction for amylin and leptin to cooperatively enhance suppression of food intake and body weight. Direct application of the combination of these peptides to the VTA of rats reduced chow intake to a greater degree than leptin alone or amylin alone; this was mediated by an augmented suppression of meal size [145]. Furthermore, doses of leptin and amylin that, on their own, were ineffective to alter feeding or body weight produced significant suppressive effects on both measures when administered in combination to the VTA [145]. The neural mechanisms underlying these effects in the VTA remain to be determined; it would be interesting to know, for example, whether the cooperative interaction relies on enhanced pSTAT3 and/or IL-6 signaling, similar to the effects in the VMN.

Given the focus of much of the amylin feeding literature on the AP, it is worth noting that much of the available evidence suggests that the AP is unlikely to support the interaction between amylin and leptin. Leptin receptors are not highly expressed in the AP [176-178], and the ability of amylin to enhance leptin-induced pSTAT3 signaling in the AP is inconsistent [165, 169]. However, recent single-cell PCR data indicate that some cells in the AP express mRNA for the components of the amylin receptor as well as the leptin receptor [179]. Co-expression of the receptors at the protein level remains to be evaluated, but certainly, this novel finding invites the question of whether a subset of AP neurons may indeed support an interaction between amylin and leptin. Still, most of the literature to date seems to indicate differing relevance of the VMN and VTA versus the AP for the interactive effect of amylin with leptin for the control of energy balance. This underscores the importance of identifying the distributed neural substrates at which amylin acts, as the specific consequences of amylin signaling appear to diverge among nuclei.

As monotherapies for the treatment of obesity have been largely ineffective to safely produce sustained and meaningful reductions in body weight [180], combination therapies – and particularly those targeting amylin – may prove to be a more successful strategy for inducing long-term weight loss [31]. Indeed, the cooperative interaction between amylin and leptin agonists for enhanced suppression of body weight has been recapitulated in humans [161, 165], indicating the possible effectiveness of this and other amylin-based combination strategies for the treatment of obesity [181]. The numerous feeding-relevant signals with which amylin can interact (see [182] for review) provide a variety of potential starting points

for basic science research to understand the mechanisms by which amylin can facilitate and enhance the actions of these other signals to promote negative energy balance.

4. Effects on cognitive and motivational processes

The majority of research to date on the physiological and behavioral roles of amylin has focused on its metabolic consequences. However, several new reports indicate that amylin may also influence aspects of cognition. This introduces the intriguing possibility that amylin-targeting pharmacotherapies may have beneficial uses beyond energy balance and glycemic control. This section will discuss the role of amylin in the cognitive deficits and neuroanatomical pathologies that characterize Alzheimer's disease (AD). In addition, although amylin's actions in the mesolimbic system have focused on its function for energy balance control, the possibility that amylin's actions in the mesolimbic system may extend to aspects of cognition and non-food-directed motivated behavior will be considered.

4.1 Amylin in Alzheimer's disease: effects on cognition and neurodegeneration

T2DM and obesity each represent a significant risk factor for the development of Alzheimer's disease (AD) and other forms of cognitive impairment [183-187]. Insulin resistance has been proposed as a mechanism connecting these diseases [188, 189], but cannot fully explain the neuropathologies associated with AD [190]. Recently, amylin has been suggested as another potential link between cognitive impairment and the glycemic dysregulation associated with T2DM and obesity [33, 191]. As mentioned previously, amylin secretion is dysregulated in T2DM. In humans, lower levels of circulating amylin are associated with cognitive deficits [192, 193], and chronic systemic administration of amylin or an amylin receptor agonist like pramlintide can improve cognitive performance in animal models of AD [192, 194]. However, increased amylin may also be involved in some of the neuropathologies associated with AD.

Some of amylin's effects in AD pathology, whether beneficial or detrimental, may be indirect. For instance, the improvements in cognition from increased amylin levels might be mediated in part by amylin's hypophagic effects, as reduced energy intake is associated with better cognitive ability (see [195, 196] for review). In terms of damaging indirect effects, amylin can induce the production of inflammatory cytokines like interleukin-6 [175, 197] that could contribute to neurodegeneration [198, 199]. However, more direct roles of amylin in AD will be considered here.

Misfolding and subsequent inappropriate aggregation of protein is a shared pathological feature in T2DM and AD [200, 201]. As discussed above, T2DM is characterized in part by amyloid deposits consisting of aggregated amylin protein in the pancreas. Correspondingly, one of the neuropathological hallmarks of AD is the presence of amyloid plaques within the brain that consist largely of misfolded amyloid beta ($A\beta$) protein. $A\beta$ is a natural product of proteolytic cleavage of amyloid precursor protein (APP). The physiological functions of APP and of $A\beta$ are not completely clear, but it is thought that APP may play a role in neural plasticity and cell growth [202]. $A\beta$ is normally cleared from the brain, but when this process fails, the protein begins to accumulate in the brain and eventually aggregates in the form of amyloid plaques. These plaques are highly cytotoxic and are considered a critical

component of AD neuropathology (see [203] for review). Interesting new findings suggest that amyloid plaques within the brain contain not only A β but also amylin. In postmortem brain tissue from patients diagnosed with AD, misfolded amylin fibrils – the precursor to amylin-based amyloid – were found in blood vessels in the brain [204]. This was not observed in tissue from non-AD controls. Furthermore, amylin and A β were found to be colocalized in the brain, but not the pancreas, of AD patients [204, 205]. Together, these results suggest that peripherally-derived amyloidogenic amylin may circulate and penetrate into the brain, where it can promote amyloid formation. Indeed, fibrils of amylin can “seed” the aggregation of A β , meaning that misfolded amylin can promote amyloidogenesis by acting as a core around which proteins such as A β (or more amylin) can aggregate [205, 206]. Several reports suggest that the detrimental effects of A β on neuronal activity and cell survival are mediated by amylin receptor signaling [207–211]. However, a recent study provides contrasting data showing that amylin receptors are not activated by A β [212], so this question remains open for debate.

Collectively, the available literature suggests that amylin is linked in some way to AD, but many questions remain. Although elevated plasma amylin is associated with cognitive benefits [193, 213], amylin can induce neurotoxicity in the central nervous system by accumulating in the brain [214], where it can promote the formation of amyloid plaques [204–206]. In trying to understand the effects of amylin on cognition and neurodegeneration, a critical consideration is whether the peptide is misfolded or not – in other words, whether it is already more susceptible to aggregation. This distinction can drastically change the effects of the peptide. For example, in a study of primary neuronal cultures, human amylin that had been “aged” to promote fibril formation had more potent neurotoxic effects than “non-aged” amylin [215]. An additional complication is that amylin is only prone to aggregation in certain species. Although it is highly amyloidogenic in humans, non-human primates, and cats [216–218], this is not the case in rodents, where a proline substitution in a key region of the peptide stabilizes the conformation to prevent fibril formation [219]. The presence of the amyloidogenic amino acid sequence alone is not sufficient to induce fibrils; increased presence of amylin in the tissue is also important for amyloid formation [220]. The species from which experimentally administered amylin is derived, and the species and conditions in which testing occurs, are undeniably important considerations in AD research.

In trying to understand the link between AD and metabolic diseases like T2DM, and particularly in trying to elucidate the role of amylin in AD, the fluctuation in amylin levels in early versus later stages of T2DM may be an important determinant of the effects of the peptide. For example, in species prone to amyloid formation, it is possible that during the earlier, hyperamylinemic stages of T2DM, the excess amylin may promote the formation of central amyloid plaques. This would be consistent with the finding that both an amyloidogenic amino acid sequence and high local amylin levels are required for misfolding and aggregation [220]. Later in the progression of T2DM, when amylin levels fall, increasing amylin via exogenous administration may have beneficial effects. The cognitive improvements associated with increased amylin levels may be due to an ability of non-amyloidogenic amylin agonists to reduce amyloid plaques in the brain. A recent study [194] examined the effects of amylin or pramlintide administration on A β levels in the brain in two separate mouse models of AD. In both models, chronic treatment with either agonist reduced

the number and size of amyloid plaques in the brain and also improved performance in two cognitive tasks [194]. Intriguingly, it appears that amylin receptor agonists may exert this effect by clearing the A β from the brain into the serum [194]. Unfortunately, the authors do not state the species from which the exogenously-administered amylin was derived, which is an important distinction. Nevertheless, one might safely conclude that the administration of a non-amyloidogenic amylin receptor agonist like pramlintide may be beneficial for the treatment of AD.

Although more research is required to elucidate the exact effects of amylin in AD, the possibility that amylin pharmacotherapies may be useful for the treatment of AD and other types of cognitive impairment is already being considered [35, 192]. Clearly, the development and application of any amylin-based pharmacological strategy for treating AD must carefully weigh the amyloidogenic potential of the amylin compounds against the possible benefits of the drug. Pramlintide is considered a viable non-amyloidogenic possibility [35], but the identification of other non-aggregating amylin receptor agonists [107] may also provide more options for drug development.

Finally, though central sources of amylin have recently been identified [54, 148, 149], the possible influence of centrally-produced amylin on AD pathology and neurodegeneration is completely untested. The fact that these sources of central amylin have been identified in rodents, in which native amylin is non-amyloidogenic, presents a hurdle. If the central sources of amylin are recapitulated in a species with amyloidogenic amylin, such as the cat – and if centrally-produced amylin, like that produced peripherally, is prone to misfolding and aggregation – this could be a useful animal model to tease apart the contributions of amylin derived from peripheral versus central sources.

4.2 Anti-psychotic effects and motivational consequences of amylin signaling: new areas of research

Although the effects of amylin on cognition have been explored most intensely in the realm of neurodegeneration and AD, possible roles for amylin in other types of cognitive processes are beginning to be considered. For example, amylin receptor activation in the mesolimbic reward system may improve deficits in sensorimotor gating, as assessed by prepulse inhibition (PPI). PPI is reduced in patients with schizophrenia [221] as well as other psychiatric conditions including obsessive-compulsive disorder [222]. The PPI paradigm has been used as a screening tool for the identification of psychiatric drugs [223, 224]. Work from Baldo and colleagues demonstrates that microinjection of amylin directly into the NAcSh of rats blocked the suppression of PPI produced by amphetamine [34], consistent with an anti-psychotic effect of amylin. This introduces a fascinating possible use for amylin-based pharmaceuticals, but more research is clearly needed to establish whether the beneficial effects observed in this single report might extend to other symptoms of psychiatric disease.

In addition, it is possible that amylin's effects in the mesolimbic reward system may impact processes related to general (i.e., non-food) motivation and cognition. Work from our laboratory demonstrates that amylin receptor activation in the VTA reduces the motivation to work for a palatable food [143]. Given the role of the VTA in motivational processes in

general, it is possible that amylin may act within the VTA to reduce other types of motivated behavior, e.g. non-food-related. A small but growing body of literature supports the notion that neurohormonal signals traditionally associated with energy balance control can act at the VTA to regulate not only feeding but also other types of motivated behavior and potentially even cognitive processing. For example, ghrelin is an orexigenic hormone produced by the stomach. Ghrelin can act directly in the VTA to increase motivation to obtain food, as assessed by operant responding [140, 225], but ghrelin signaling in the VTA also affects sexual motivation in mice [226]. Additionally, the effects of VTA ghrelin extend to cognitive processes, as ghrelin receptor activation in this nucleus increases impulsive-like behavior in rodents [227]. This provides precedence for the idea that the actions of feeding-related peptides in the VTA may exert effects on motivation and cognitive processes beyond outcomes relevant for feeding. The notion that amylin signaling in the VTA might also have broader consequences for motivation and cognition is an exciting possibility that remains to be addressed empirically.

5. Conclusion

T2DM and obesity are costly diseases that affect millions of people worldwide. Pharmacotherapies to address each of these conditions are available, but effective and non-invasive options are limited, particularly for the treatment of obesity. The amylin analog pramlintide is currently FDA-approved as an adjunctive therapy for diabetes, demonstrating the proven clinical relevance of amylin-based drugs for the treatment of this disease. The amylin system also has excellent potential for use in the treatment of obesity. Amylin exerts potent suppressive effects on food intake and body weight, and can interact with other neuroendocrine signals to produce cooperative hypophagic effects, broadening its possible clinical applications. Given the frequent comorbidity of diabetes and obesity [228], developing pharmacotherapies that can effectively treat both diseases simultaneously would be beneficial and perhaps improve patient compliance [229]. The robust and well-established ability of amylin-based compounds to improve glycemic control and to promote negative energy balance make this hormonal system a likely candidate.

Recent research also suggests that amylin may exert effects on cognition. In general, higher amylin levels are associated with improvements in cognitive ability. Numerous reports have investigated the role of amylin in the etiology of AD; although many questions remain, it appears that exogenous administration of amylin may improve the neurodegenerative and cognitive effects of AD [194]. This type of treatment has not been tested in humans, but if effective, it might represent a new strategy to treat AD and other types of cognitive impairment. Furthermore, given the association of T2DM and obesity with AD, it is possible that amylin pharmacotherapies could also provide beneficial treatment for this neurodegenerative disease in patients with these types of comorbidities. Compared to the well-established effects of amylin on glycemic regulation and energy balance control, and the clear benefits of pramlintide for regulating postprandial blood glucose levels, research on its impact on cognition and any potential utility for the treatment of AD is in its infancy. Nevertheless, the amylin system may hold broader promise for the treatment of neurodegenerative and psychiatric disorders. This lively area of research will assuredly

produce many exciting findings that may lead to new translational and pharmacological possibilities for amylin.

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Highlights

- Amylin is physiologically relevant for glycemic and energy balance control
- Amylin receptor agonists reduce blood glucose, feeding, and body weight
- Amylin may reduce cognitive and neurodegenerative symptoms in Alzheimer's disease
- Amylin may exert anti-psychotic effects
- Amylin-based pharmacotherapies may provide benefit to metabolic/cognitive outcomes