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Amylin activates distributed CNS nuclei to control energy balance

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Abstract

Amylin is a pancreas-derived neuropeptide that acts in the central nervous system (CNS) to reduce food intake. Much of the literature describing the anorectic effects of amylin are focused on amylin's actions in the area postrema, a hindbrain circumventricular structure. Although the area postrema is certainly an important site that mediates the intake-suppressive effects of amylin, several pieces of evidence indicate that amylin may also promote negative energy balance through action in additional CNS nuclei, including hypothalamic and mesolimbic structures. Therefore, this review highlights the distributed neural network mediating the feeding effects of amylin signaling with special attention being devoted to the recent discovery that the ventral tegmental area is physiologically relevant for amylin-mediated control of feeding. The production of amylin by alternative, extra-pancreatic sources and its potential relevance to food intake regulation is also considered. Finally, the utility of amylin and amylin-like compounds as a component of combination pharmacotherapies for the treatment of obesity is discussed.

Keywords

IAPP; obesity; pramlintide; VTA; reward

1. Introduction

Amylin, also known as islet amyloid polypeptide (IAPP), is a 37-amino acid peptide hormone that is produced by pancreatic β-cells and co-secreted with insulin in response to the presence of nutrients in the gastrointestinal tract [1, 2]. Amylin has several physiological functions, but perhaps the most well-studied amylin-mediated effects are those of reduced food intake and improved glycemic control [3, 4]. In fact, the amylin analog pramlintide (Symlin) is FDA-approved for the treatment of diabetes mellitus [5], but has the additional effect of producing body weight loss in humans [6]. The actions of amylin to suppress food intake and body weight will be the focus of this review.

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Amylin acts centrally to control energy balance, and this effect has been attributed primarily to activation of amylin receptors on neurons of the area postrema (AP), a hindbrain circumventricular structure. The evidence supporting the AP as an important amylin-responsive site has been extensively reviewed [2, 7, 8] and is discussed briefly below. However, the neural control of energy balance is not restricted to a select nucleus or subset of nuclei, but rather is distributed across the entire CNS [9, 10]. Given that amylin binds to sites throughout the CNS [11-15], the idea that amylin may act at nuclei other than the AP to control feeding has received surprisingly little attention. Recent data from our laboratory [16] and others [17-20] have begun to highlight the importance of additional CNS structures in contributing to the intake- and body weight-suppressive effects of amylin. Specifically, we have recently shown that the ventral tegmental area (VTA), a mesolimbic nucleus important in food intake, food reward, and motivated behavior [21-23], is a physiologically relevant site for the actions of amylin to reduce food intake [16]. Thus, the ability of amylin to promote negative energy balance through activation of extra-AP sites is the main focus of this review.

2. Amylin receptors: physiology and location

2.1 The amylin receptor complex

The amylin receptor (AMY) comprises a calcitonin receptor (CTR) heterodimerized with a receptor activity modifying protein (RAMP). There are two splice variants of CTR, named CTR_A and CTR_B. CTR_A is the more prevalent isoform and is widely expressed in the periphery and the brain [24]. CTR_B expression is restricted to the CNS, but at lower levels than CTR_A [12, 24]. The CTR can be activated by several closely related peptides including calcitonin, amylin, adrenomedullin, and CGRP. The CTR alone has the highest affinity for calcitonin [25, 26], but enhanced selectivity for amylin binding is conferred by association with a RAMP [27, 28], of which there are three possible subtypes for the amylin receptor complex (RAMP1, RAMP2, RAMP3) [29]. Together with the two splice variants of CTR, this totals six unique CTR/RAMP combinations that can form a functional amylin receptor [30], although RAMP1 and RAMP3 may confer greater amylin binding selectivity than RAMP2 [27, 28]. Amylin receptor nomenclature therefore reflects the unique combination of RAMP and CTR constituting the amylin receptor complex (e.g., AMY_{1A} indicates a heterodimer of RAMP1 and CTR_A) [30].

The CTR is a G_s/G_q-protein-coupled receptor [24, 31, 32]. This G-protein coupling remains intact when the CTR/RAMP heterodimer forms, reflected by the finding that amylin receptor activation increases cyclic AMP (cAMP) and intracellular calcium [27, 31, 33, 34]. Cyclic GMP (cGMP) may also be an important second messenger of amylin receptors, at least in the AP, as peripheral administration of amylin increases cGMP levels in this nucleus [35]. The downstream effects of G-protein pathways are often numerous and diverse [36], but the specific intracellular signaling molecules engaged by amylin receptor activation have only recently begun to be investigated and are discussed below in greater detail.

Much of the previous research characterizing the structure and second messenger systems of amylin receptors has utilized the human isoforms of CTRs and RAMPs [25, 27, 28, 31], but recently, the ligand binding and receptor activation profiles of rat AMY_{1A} and AMY_{3A}

receptors were characterized *in vitro* [33]. The components of rat and human amylin receptors have relatively high sequence homology [37, 38], and in many ways, rat amylin receptors recapitulate previous data obtained from studies of human amylin receptors. Specifically, the combination of rat CTR_A with rat RAMP1 or RAMP3 had greater affinity for amylin than did rat CTR_A alone, and rat amylin receptors were more potently activated by amylin than other related peptides [33]. However, some differences between rat and human amylin receptors were revealed; for example, the affinity of the rat AMY_{3A} receptor for CGRP is higher than that reported in previous studies of the human AMY_{3A} receptor [33, 39]. The characteristics of rat amylin receptors containing RAMP2 or CTR_B were not analyzed; this would be a useful follow-up study. Also of importance is the fact that this study of rat amylin receptors was conducted in a kidney-derived cell line [33]; given that cellular background can influence the function of human amylin receptors [28], future characterization of both human and rat amylin receptors in a neuronal cell line, as well as *in vivo*, may be especially informative.

2.2 Amylin binding in the CNS

Amylin binding has been observed in numerous sites throughout the CNS. These include, but are not limited to, hindbrain sites such as the AP and nucleus of the solitary tract (NTS), hypothalamic subnuclei including the lateral and ventromedial hypothalamic nuclei, as well as mesolimbic structures such as the nucleus accumbens (NAc) and ventral tegmental area (VTA) [11-13, 40, 41]. To date, much of our knowledge of CNS amylin binding is derived from autoradiography experiments, in which radiolabeled amylin or amylin agonists are applied directly to brain slices. These studies are informative as to the location of functional amylin binding sites, but do not identify the endogenous source providing amylinergic input to these CNS nuclei. Based on our current knowledge, pancreatic amylin is a likely candidate. Indeed, a study in mice showed that when injected intravenously, radiolabeled amylin can cross the blood-brain barrier and accumulate in several CNS sites, including cortical areas, the hypothalamus, and the striatum [15], suggesting that peripheral amylin can access the CNS. However, it is also possible that other source(s) of amylin could activate CNS amylin-binding nuclei. The potential existence of alternative, non-pancreatic sources of amylin is discussed in more detail in a later section.

There appears to be some degree of site specificity in the expression of the amylin receptor subtypes. The AP expresses primarily AMY_{3A} [42], whereas the NAc expresses predominantly AMY_{1A} [37]. In contrast, although CTR_A is the prevalent CTR subtype in the VTA, all three subtypes of RAMP are expressed at similar levels within the VTA [16]. Our knowledge of site-specific amylin receptor subtype expression has been derived from techniques probing CTR and/or RAMP mRNA expression within a given nucleus [16, 37, 42]. Although the presence of CTR and RAMP expression along with site-specific amylin binding provide solid converging evidence supporting the presence of amylin receptors, the expression and association of specific CTR and RAMP subtypes at the single-neuron level within nuclei that bind amylin is unknown. Future studies are therefore needed to assess the coexpression and colocalization of CTRs and RAMPs within single cells in CNS nuclei of relevance to amylin's effects on energy balance regulation. Such research may in turn provide important foundational evidence for the evaluation of the neurochemical phenotypes

of amylin receptor-expressing neurons, especially in sites such as the VTA that express multiple RAMP subtypes [16].

3. Amylin activates the area postrema to reduce food intake

Although amylin is produced predominantly by the pancreas, a central site of action for the hormone is suggested by the finding that neither surgical vagotomy [43] nor chemical ablation of peripheral sensory afferent fibers via capsaicin treatment [44] attenuates the ability of peripherally administered amylin to reduce food intake. As mentioned briefly above, the most well-studied CNS site mediating the intake-suppressive effects of amylin is the area postrema (AP), a hindbrain circumventricular structure. Numerous pieces of evidence support the idea that the AP is a pharmacologically and physiologically relevant site for the anorectic actions of amylin. Microinjection of amylin or the amylin receptor agonist salmon calcitonin directly into the AP of rats reduces food intake [45]. Furthermore, a physiological role for the AP in amylin-mediated regulation of food intake is supported by data showing that intra-AP injection of the amylin receptor antagonist AC187 increases feeding [45], suggesting that endogenous amylin acts in the AP to control energy balance. Activation of the AP by systemic amylin has been demonstrated by c-Fos expression [17, 46]. Peripherally administered amylin also induces c-Fos in other nuclei of the visceral afferent pathway thought to be downstream of AP amylin receptor activation, including the nucleus of the solitary tract (NTS), lateral parabrachial nucleus (lPBN), and central nucleus of the amygdala (CeA) [17]. The use of electrolytic lesions has provided further evidence for the role of the AP in the anorectic effects of amylin. Lesion studies indicate that the ability of peripherally administered amylin or amylin agonists to suppress feeding is blunted in AP-lesioned rats [47-49]. In addition, the induction of c-Fos in the NTS, lPBN, and CeA produced by systemic amylin treatment is attenuated by AP lesions [17]. Though the results of these experiments complement data obtained using other experimental techniques, collectively indicating the importance of the AP in mediating amylin-induced hypophagia, there are characteristics of the AP lesion model that must be taken into consideration when interpreting these studies.

Lesioning the AP produces alterations to normal energy homeostasis. Rats without an intact AP have moderate reductions in daily food intake compared to sham-lesioned control animals, resulting in lower daily body weight gain [47]. An additional consideration in an AP-lesioned rat is the potential disruption of neuronal processing that originates in other CNS structures but involves secondary processing by the AP. For example, the NTS is a site both adjacent to and closely associated with the AP; dendritic and axonal processes of neurons from one site extend into the other [50-52]. As the NTS represents a key CNS hub that processes and integrates several neural and hormonal signals relevant to energy balance [53, 54], and is also an amylin binding site [13, 40], it is unclear how the AP lesion might impact any effects amylin may exert through NTS activation. That is, the AP lesion disrupts NTS-to-AP and AP-to-NTS neural pathways, as well as similar neural pathways from other CNS nuclei that also communicate monosynaptically with the AP. The disturbance of these afferent and efferent AP neuronal processes may therefore contribute to the observed effects of AP lesions with regard to dysregulation of normal energy balance control and the attenuated ability of amylin to reduce food intake. These points are not made to dispute the

importance of the AP as a site mediating the anorectic effects of amylin, but rather to encourage detailed consideration of the broader implications of the AP lesion studies, as well as the possibility that other CNS structures may have a role in amylin-induced hypophagia.

4. Alternative CNS sites of action for amylinergic control of energy balance

Although a multitude of studies have demonstrated convincingly that the AP is an important site mediating the effects of amylin on energy balance [17, 45, 55], the collective body of literature should not be interpreted to mean that the AP is the only site at which amylin acts to exert these effects. Importantly, even in rats with AP lesions, amylin- and amylin agonist-mediated behavioral and physiological effects usually attributed to AP amylin receptor activation are still observed. For example, AP-lesioned rats still exhibit an amylin-induced suppression of food intake under carefully controlled experimental conditions [47]. Additionally, although AP lesions blunt c-Fos expression in sites downstream of AP amylin receptor activation (NTS, lPBN, CeA), amylin-induced c-Fos in these sites is not completely abolished by an AP lesion [17]. It is possible that these residual effects are attributable to incomplete AP lesions, but another interpretation of these data would suggest that brain regions other than the AP may also mediate the anorectic effects of amylin on food intake.

4.1 Hypothalamic actions of amylin

In comparison to the vast amount of research exploring amylin's actions in the AP, a paucity of studies have investigated potential amylin activation of extra-AP CNS structures to control food intake. Several subnuclei in the hypothalamus bind amylin [13, 41], and peripherally administered radiolabeled amylin can cross the blood-brain barrier to accumulate in the hypothalamus [15]. Furthermore, two studies have shown that intra-hypothalamic administration of amylin reduces food intake in rats [20, 56], although extremely high doses of amylin were used in these studies, suggesting that the data should be interpreted with caution. Collectively, however, the available data hint at the possibility that direct amylin receptor activation in one or more hypothalamic nuclei may mediate in part amylin's effects on food intake. The hypothalamus has been further implicated in amylin-mediated reduction of feeding by the finding that peripherally administered amylin induces changes in gene expression of key hypothalamic feeding-related neuropeptides, including increased mRNA for the anorexigenic neuropeptide preopiomelanocortin (POMC) in the arcuate nucleus (Arc) [19] and reduced mRNA for the intake-promoting neuropeptide orexin in the lateral hypothalamus (LH) [57]. Both of these changes likely contribute to overall amylin-mediated reductions in feeding; however, as amylin was administered systemically in these studies, it remains an open question whether these effects are due to direct actions of amylin at the hypothalamus or indirect, downstream consequences of amylin binding at a non-hypothalamic CNS site. Clearly, more research designed to tease apart the direct and indirect contributions of the hypothalamus to amylin-mediated control of feeding is urgently needed.

Previous studies have sought to characterize the role of the LH in the anorectic effects of amylin. First, Riediger et al. demonstrated that fasting-induced c-Fos expression in the LH is attenuated by intraperitoneal amylin injection [17]. More recently, a neuronal tracing study

showed that amylin-activated neurons in the lPBN densely and directly project to the LH [58]. Because peripheral amylin treatment appears to induce c-Fos in the lPBN in part through AP amylin receptor activation [17], this lPBN-LH projection may be a secondary response downstream of AP amylin receptor stimulation. However, given that the LH binds amylin receptor agonists in autoradiographic analyses [13] and exhibits CTR-like immunoreactivity [59], a direct action of amylin at the LH should not be ruled out.

4.2 Amylin signaling in the mesolimbic reward system

While exploration of amylin's direct action in the hypothalamus has been sparse in comparison to the literature looking at the AP as the primary site mediating amylin's anorectic effects, research evaluating extra-hypothalamic and extra-brainstem CNS sites as potential direct mediators of amylin's energy balance effects is even sparser. An analysis of the literature for energy balance-relevant nuclei that also express components of the amylin receptor complex and/or display amylin or amylin agonist binding focuses attention on nuclei of the mesolimbic reward system. Indeed, the nucleus accumbens (NAc) is a key mesolimbic structure implicated in food intake and reward [60] that expresses the CTR [16, 59] and exhibits robust amylin binding in autoradiography studies [11, 40]. Yet, despite these interesting findings, only one paper to date has examined a potential function for amylin acting in the NAc to suppress feeding [61]. The data indicated that direct injection of amylin into the NAc shell of food-deprived rats reduced food intake, whereas NAc core injections of amylin had no significant effect on feeding [61]. However, a similar anorectic effect of intra-NAc shell amylin was observed in water-deprived rats only when the placement of the guide cannula traversed the lateral ventricle; when an angled cannula placement was utilized to avoid potential ventricular diffusion of amylin, there was no longer a significant amylin-mediated reduction of food intake [61]. Unfortunately, the authors did not report the effects of intra-NAc shell amylin delivery in ad libitum fed or food-deprived rats using the angled cannula placement, leading to the possibility that the observed effects may be due to dispersion of injected amylin throughout the ventricular system and its subsequent action at other nuclei. Thus, it stands to reason that further detailed analyses of the potential intake-suppressive effects of amylin at the NAc are needed.

In addition to the NAc, the VTA is another mesolimbic site that until recently had not received any attention in the amylin literature despite the fact that CTR-like immunoreactivity [59] and *ex vivo* binding of amylin and amylin receptor agonists were reported in the VTA [13]. As many signals of energy availability act in the VTA to control food intake and food reward, including leptin [62, 63], glucagon-like peptide-1 [64-66], and ghrelin [67, 68], the VTA seemed to be a logical candidate for physiological and/or pharmacological relevance of amylin for food intake control. Indeed, our laboratory recently showed that activation of VTA amylin receptors by the agonist salmon calcitonin dose-dependently suppressed intake of chow for up to 24h at doses subthreshold for effect when delivered to the 3rd cerebroventricle. The amylin-induced hypophagia was mediated mainly by reductions in meal size, rather than meal number [16], consistent with the role of amylin as a satiation signal [69]. Importantly, these intake-suppressive effects occurred in the absence of nausea/malaise or sustained alterations in locomotor activity [16]. It was also

determined that VTA amylin receptors are physiologically relevant for the control of feeding, as intra-VTA delivery of the amylin receptor antagonist AC187 increased 24h chow intake [16].

The VTA plays an important role in the regulation of food reward (see [21, 22] for review). To assess whether VTA amylin receptor activation decreases motivation to obtain a palatable food, rats were trained to lever-press for sucrose pellets on a progressive ratio schedule of reinforcement. Activation of VTA amylin receptors reduced active lever presses, total number of sucrose pellets earned, and breakpoint, demonstrating a reduction in the motivation to work to obtain the palatable reinforcer [16]. Taken together, our findings provided the first evidence that the VTA was a pharmacologically and physiologically relevant site of action for the anorectic effects of amylin. Given the role of VTA dopamine signaling in the control of food reward and motivated behavior [21-23], amylin receptor activation may reduce food intake by modulating dopaminergic signaling. This is an especially interesting possibility given the finding that central amylin administration reduced the frequency of copulation in male rats [70], suggesting that amylin may also exert suppressive effects on sexual behavior. The generalizability of these findings should be assessed by testing the ability of amylin to decrease other motivated behaviors.

5. An alternative endogenous source of amylin?

If the amylin produced and secreted by the pancreatic β-cells represents the sole source of endogenous amylin in the body, it seems logical to assume that pancreas-derived amylin must travel humorally to gain access to the brain. The fact that the AP is not protected by the blood-brain barrier provides a parsimonious explanation for how amylin reaches this circumventricular structure. However, the finding that VTA amylin receptors are also physiologically relevant for the control of food intake leads to the question of how endogenous amylin is able to access the VTA, and potentially other CNS amylin-responsive sites that are not circumventricular nuclei. Two non-exclusive possibilities exist. First, the penetrance and facilitated/active transport of amylin into the brain may be fairly easy and robust. This notion is quite plausible, given the small size of amylin (37 amino acids) as well as the ability of exogenous, peripherally administered amylin to cross the blood-brain barrier [15, 71] and to bind to an abundant variety of CNS nuclei. Second, an alternative source of amylin may exist within CNS neurons that project to a multitude of CNS structures, including the VTA.

Amylin-like immunoreactivity and/or mRNA expression has been observed in the gastrointestinal tract [72, 73] and the dorsal root ganglia [74], indicating alternative, non-β-cell tissues with the genetic machinery present to be potentially capable of producing functional amylin protein. Recent studies have also shown that mRNA for amylin is upregulated in the brain of maternal female rats. More specifically, amylin mRNA is expressed in the preoptic area (POA) of maternal dams [75], as well as concaveated nulliparous female rats [76], but has not been detected in the POA of non-maternal virgin females [76] or male rats [75]. These findings suggest a specific association of POA amylin with maternal behavior. Although this is the only condition under which amylin production has been observed in the brain thus far, it hints at the intriguing possibility that endogenous

amylin may be present in the CNS under other specific behavioral conditions, or in sites that have not yet been examined. Importantly, the relative contribution to energy balance regulation, if any, of the amylin produced by these putative alternate sources remains to be determined.

6. Intracellular and neurochemical mediators of amylin-induced suppression of intake

6.1 Amylin receptor-mediated intracellular signaling pathways

The activation of a G-protein coupled receptor, such as the amylin receptor, by binding of its ligand activates or inhibits numerous intracellular signaling molecules [36]. Broadly speaking, stimulation or suppression of these intracellular pathways can impact a variety of physiological processes that alter behavior. Activation of specific intracellular signaling molecules can induce changes both at the cellular level (e.g., alterations to gene expression; changes in intracellular calcium signaling; neuronal glucose utilization) and at the synaptic level (e.g., alterations in neuronal plasticity) [77-80]. Intracellular signaling pathways also serve as points of convergence for the integration of neurally- and humorally-mediated signals important for energy balance control and other physiological processes [54, 81]. Thus, by furthering our understanding of the intracellular mechanisms engaged by a particular receptor, we gain insight into subsequent changes mediated by downstream intracellular signaling molecules that may greatly impact behavior. The intracellular signaling cascades engaged by amylin receptor activation and the relevance of these signals to amylin-mediated changes in energy balance are only beginning to be examined.

In the Arc, peripheral amylin treatment marginally increases phospho-STAT3 [82], an effect generally consistent with observations in the GT1-7 hypothalamic neuronal cell line [83]. The requirement of this increase in STAT3 activation for changes in feeding behavior has not yet been determined. On the other hand, the requirement of phosphorylated extracellular-signal regulated kinase 1/2 (ERK1/2, also known as MAPK) in the AP for amylin-induced hypophagia is clearer. Immunohistochemical techniques show that phospho-ERK1/2 is induced in AP CTR-expressing neurons after subcutaneous amylin administration [84]. Critically, the behavioral relevance of this intracellular signaling molecule was revealed by the finding that 4th intracerebroventricular pretreatment with the MEK1/2 inhibitor U0126 (thus inhibiting phosphorylation of ERK1/2) attenuated the food intake-suppressive effect of peripheral amylin [84]. To date, this particular effect has only been tested in the AP; whether an increase in ERK1/2 phosphorylation also mediates the reduction in food intake produced by amylin receptor activation at other CNS sites remains to be tested. It is also worth noting that the second messenger cGMP may be associated with amylin-induced reduction of food intake in the AP; peripheral amylin induces cGMP formation in this nucleus [35] and microinjection of a cGMP mimetic into the AP suppresses food intake [45]. The potential *requirement* of cGMP formation for the anorectic effects of amylin remains to be investigated. Future experiments should also assess *in vivo* amylin-mediated induction of other second messengers and intracellular signaling molecules identified by *in vitro* studies, and the potential relevance of these changes for the effects of amylin on feeding.

6.2.1 Neurochemical mediators of the anorectic actions of amylin: histamine—

As the intracellular signaling molecules required for the anorectic actions of amylin continue to be identified, another focus of amylin research has been to pinpoint other neurochemical systems that are engaged by amylin to suppress feeding. One of the first neurochemicals implicated in the hypophagic effect of amylin was histamine [85]. Intraperitoneal injection of drugs to block histaminergic transmission, at doses that alone had no effect on feeding, reduced the ability of amylin to suppress food intake [85]. Given the role of central histamine, and especially hypothalamic H1 histaminergic receptors, in the control of feeding [86, 87], a follow-up study used H1 receptor-knockout mice to demonstrate that the ability of amylin to suppress feeding was blocked in the absence of H1 receptors [88]. Later studies focused on the role of the ventromedial hypothalamus (VMH) in this effect, as peripheral amylin increases histaminergic signaling in the VMH [89], and VMH H1 receptor blockade attenuates the hypophagic action of systemic amylin [18]. Whether these changes in histaminergic signaling may also mediate amylin-induced reductions in food intake in other CNS sites remains to be determined.

6.2.2 Neurochemical mediators of the anorectic actions of amylin: catecholamines—

Catecholaminergic (dopamine and norepinephrine) signaling is also implicated as a mediator of the feeding-suppressive effects of amylin. The robust expression of noradrenergic neurons in the AP and adjacent NTS [90] suggests the potential involvement of norepinephrine in amylinergic signaling. Indeed, Potes et al. confirmed the role of hindbrain noradrenergic neurotransmission in the intake-suppressive effects of amylin [55]. First, an immunohistochemical analysis revealed that a subset of AP neurons activated by peripheral injection of amylin were noradrenergic [55]. As mentioned earlier, peripheral amylin treatment increases ERK1/2 activation in the AP; at least some of these phospho-ERK-positive neurons were noradrenergic [84], suggesting changes in ERK1/2 as a possible link between amylin receptor activation and alterations in norepinephrine neurotransmission. The physiological importance of hindbrain noradrenergic neurons for amylin-mediated control of food intake was tested by using a selective lesion to destroy norepinephrine neurons in the AP and lPBN. This manipulation attenuated the ability of peripheral amylin to reduce feeding [55], supporting the notion that these hindbrain noradrenergic neurons are required for the full intake-suppressive effects of amylin. Yet, this experimental technique does not only disrupt noradrenergic signaling but in fact destroys the noradrenergic cells, thereby presumably disrupting signaling by other neurochemicals co-secreted along with norepinephrine from these neurons. Thus, further pharmacological studies are needed to confirm the specific requirement of norepinephrine for the anorectic action of peripherally administered amylin, as well as to elucidate the potential contribution of other transmitters released by the noradrenergic neuronal population in this effect.

Normal dopaminergic receptor signaling is also required for the hypophagic effects of amylin [91]. Specifically, blockade of dopaminergic D2 receptors with systemic raclopride, but not blockade of D1 receptors, attenuated the ability of intraperitoneal amylin to reduce food intake [91], suggesting a role for dopamine signaling in the amylin-mediated response. It is not yet known, however, whether amylin modulates D2 receptor activation directly through an increase in dopamine signaling, or whether the raclopride effects reported are

due to modulation of a downstream polysnaptic response to amylin. Furthermore, additional complexity is conferred by the fact that D2 receptors can act as either postsynaptic receptors or presynaptic autoreceptors [92], leading to the question of which receptor population is relevant for amylin-induced intake suppression. The aforementioned study [91] highlights the importance of D2 receptors, but the systemic administration of raclopride leaves open the question of the CNS site(s) mediating the effect. The recent finding that activation of VTA amylin receptors can suppress food intake [16], coupled with the fact that dopaminergic neurons are highly expressed within the VTA [93, 94], suggests the possibility that alterations in VTA dopaminergic signaling may contribute to amylin-induced hypophagia.

7. Amylin acts cooperatively with other feeding-related peptides to suppress food intake and body weight gain

7.1 Amylin-based combination pharmacotherapies for obesity treatment

The incidence of obesity has risen dramatically in many countries around the world [95, 96]. Effective anti-obesity drugs are urgently needed as behavioral therapy offers limited success, and although gastrointestinal bariatric surgery is effective, it can have serious adverse consequences [97]. To this end, pharmaceutical treatments targeting the amylin system may hold promise for treating obesity. Amylin receptor agonists reduce food intake and body weight in both humans and animal models [19, 98]. Although the idea that amylin-based combination therapies may hold promise for treating obesity has gained traction in the literature over the last few years, it is critical to note that no mono-drug therapy to date has been universally effective to treat obesity. Indeed, the idea that combination therapies will yield more effective treatment options for obesity, compared to lifestyle modifications or mono-drug therapies alone, has become increasingly accepted [99, 100]. Thus, greater progress in developing effective anti-obesity drugs may result from research aimed at identifying common neural circuits mediating the food intake-inhibitory effects of multiple physiological signals.

Recent literature has demonstrated that amylin can interact with other feeding-related peptides to produce additive or synergistic reductions in food intake and body weight gain. Cooperative interactions have been observed between an amylin receptor agonist and other anorexigenic signals including leptin [82, 101, 102], cholecystokinin [103, 104], glucagon-like peptide-1 [105], and melanocortin-4 [106], among others. However, the mechanisms by which amylin interacts with these other peptides to produce enhancements in the suppression of food intake and body weight are largely unknown at this point. These interactions are likely mediated by downstream, coordinated effects of the peptides. For example, receptor signaling may converge on shared intracellular signaling pathways to alter neuronal activity and/or subsequent neurotransmission in a cooperative manner; however, as detailed below, we are only beginning to identify and understand these mechanisms. The combination of amylin and leptin will be the focus of this section; for discussion of additional amylin-based combinations, see other recent reviews [107, 108].

7.2 Amylin acts cooperatively with leptin to promote negative energy balance

Leptin is an adipose tissue-derived hormone that, like amylin, reduces food intake and body weight [109, 110]. However, the ability of exogenous leptin to promote negative energy balance is greatly diminished in the obese state [111, 112]; amylin receptor agonists, in contrast, remain effective to reduce food intake and body weight in obese rodents [101, 113] and overweight/obese humans [114, 115]. The cooperative interaction between amylin and leptin has been especially intriguing in the context of obesity treatment, because amylin appears to restore sensitivity to leptin in the obese state [101, 102]. Therefore, several recent publications have explored the ability of the combination of amylin and leptin to enhance the suppression of food intake and body weight, and either additive or synergistic effects have been reported depending on the particular experimental conditions [102, 116-119].

One of the first pieces of evidence for an interaction between amylin and leptin came from a mouse study in which peripheral administration of a combination of the amylin receptor agonist sCT and leptin produced an additive enhancement in the reduction of food intake in lean, chow-fed mice [116]. This general response was observed subsequently under several different permutations, including in lean rats [82, 117]; different routes of drug administration [117]; maintenance on a palatable high-fat diet [102]; and chronic rather than acute drug administration [101, 102, 118]. The enhancement of weight loss produced by the amylin + leptin combination is partly mediated by the increased suppression of food intake; however, pair-feeding experiments demonstrate that hypophagia alone cannot account for the enhanced degree of body weight loss produced by the combination of the two hormones [101]. Indeed, rats treated with amylin + leptin have reduced adiposity due to enhanced fat utilization, as evidenced by sustained reductions in the respiratory exchange ratio (RER) [102]. Hepatic mRNA expression of several genes related to lipid metabolism is also altered by the amylin + leptin combination, with the overall effect of reducing lipogenesis [102]. These alterations in adiposity and lipid metabolism likely contribute to the enhanced effect of the combination over pair-feeding alone.

Hypothalamic subnuclei including the VMH and Arc appear to support, at least in part, the cooperative interaction of amylin and leptin. Acute peripheral administration of the combination of amylin and leptin increased pSTAT3 expression in the Arc of lean rats more than either peptide alone [82]. In diet-induced obese, leptin-resistant rats, chronic amylin administration was able to restore the leptin-induced induction of pSTAT3 in the VMH [101]. The restoration or enhancement of leptin signaling in these sites by amylin may be due to increased leptin binding when these peptides are administered in combination [82].

The role of the AP in the interaction of amylin and leptin is less clear. In a study in diet-induced obese rats, leptin alone had no effect on pSTAT3 levels in the AP, but increased the amount of AP pSTAT3 induced by amylin [101]. However, in lean rats, no such enhancement occurred [82]. This discrepancy could be related to the obesity or dietary status of the rats, or methodological differences in drug dosages between the studies. Moreover, the relevance of these changes in AP pSTAT3 to reductions in food intake and body weight is unknown. Given the recent observation that direct infusion of leptin into the AP of rats does not produce significant body weight loss or hypophagia [120], it is possible that the AP is not a critical site for the interaction between amylin and leptin, and that actions of these

hormones at other CNS structures are more responsible for the cooperative effect. Further studies are clearly needed to determine the contribution, if any, of the AP in mediating this cooperative effect.

The effects of the combination of amylin + leptin on intracellular signaling molecules other than pSTAT3, as well as the downstream neurochemical mediators of this interaction, are just beginning to be tested. Experiments in adipose- and neuronal-derived cell lines recapitulate the *in vivo* finding of enhanced pSTAT3 when leptin and amylin are delivered in combination [83, 121] and also demonstrate effects on other intracellular signals. Specifically, activation of AMPK, Akt, and ERK1/2 were also increased in an additive fashion by amylin + leptin compared to either peptide alone [83, 121]. Given that ERK1/2 is required for the full intake-suppressive effects of peripheral amylin [84] and is also activated by leptin [122], ERK1/2 seems to be a promising candidate molecule that may contribute to the interactive effects of amylin and leptin to promote negative energy balance. Recently published data also indicate that histaminergic signaling contributes to the hypophagic response after administration of the combination of amylin + leptin [89], but more investigation into the contribution of other neurotransmitter systems (e.g. dopamine) is also warranted.

8. Conclusion

While the AP undoubtedly mediates some of the food intake-suppressive effects of amylin, the distributed nature of the control of energy balance [9, 10] and the multitude of CNS sites that bind amylin point to the likelihood that amylin can also reduce feeding through actions at other CNS nuclei. The recent data from our laboratory showing that amylin receptor signaling in the VTA is physiologically relevant for the control of food intake [16] are among the first published data indicating a physiological role of amylin receptors for energy balance regulation in a nucleus other than the AP. Clearly, more research is needed to identify other CNS nuclei that mediate amylin-induced changes in feeding. In addition, the finding that VTA amylin receptor activation reduces the motivation to work for a palatable food [16] demonstrates a previously unknown role for amylin in regulating food reward, and also hints at the intriguing possibility that amylin may also act in the mesolimbic reward system to reduce other motivated behaviors. Given the putative utility of amylin-based combination pharmacotherapies for the treatment of obesity [107, 108], a fuller understanding of the CNS actions of amylin is urgently needed. Many unanswered questions remain to be explored, and indeed, we may be in the beginning stages of a new understanding of amylin-mediated control of energy balance.

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