Review

MOLECULAR METABOLISM

Amylin — Its role in the homeostatic and hedonic control of eating and recent developments of amylin analogs to treat obesity

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ABSTRACT

Background: Amylin is a pancreatic β-cell hormone that produces effects in several different organ systems. One of its best-characterized effects is the reduction in eating and body weight seen in preclinical and clinical studies. Amylin activates specific receptors, a portion of which it shares with calcitonin gene-related peptide (CGRP). Amylin's role in the control of energy metabolism relates to its satiating effect, but recent data indicate that amylin may also affect hedonic aspects in the control of eating, including a reduction of the rewarding value of food. Recently, several amylin-based peptides have been characterized. Pramlintide (Symlin®) is currently the only one being used clinically to treat type 1 and type 2 diabetes. However other amylin analogs with improved pharmacokinetic properties are being considered as anti-obesity treatment strategies. Several other studies in obesity have shown that amylin agonists could also be useful for weight loss, especially in combination with other agents.

Scope of review: This review will briefly summarize amylin physiology and pharmacology and then focus on amylin's role in food reward and the effects of amylin analogs in pre-clinical testing for anti-obesity drugs.

Conclusion: We propose here that the effects of amylin may be homeostatic and hedonic in nature.

Keywords Amylin; CGRP; Homeostatic; Hedonic; Reward; Analog

1. AMYLIN IN THE CONTROL OF ENERGY METABOLISM

The pancreatic hormone amylin is co-synthesized and co-released with insulin from pancreatic beta-cells [1—3]. It has long been thought that pancreas-derived amylin is the only relevant source of amylin to control metabolism. However, recent evidence has shown that amylin is also expressed in the central nervous system, in particular in parts involved in metabolic control, such as the lateral hypothalamus (LH) [4]. Globally, amylin controls nutrient fluxes by reducing energy intake, modulating nutrient utilization and increasing energy expenditure. The best-investigated function of amylin is its role as satiation signal. Indeed, chronic administration of amylin reduces total energy intake, which eventually results in the reduction of body weight [5,6]. These findings were the basis for the development of amylin analogs that may represent a new approach for the treatment of overweight in obese individuals [7,8].

The caudal hindbrain area postrema (AP) and nucleus of the solitary tract (NTS) are critically involved in mediating the effects of amylin on eating [9]. However, recent data indicate that other areas of the brain, including the hypothalamic arcuate (ARC) or ventromedial (VMN) nucleus [10,11], ventral tegmental area (VTA) [12,13], and lateral dorsal tegmental nucleus (LDTg) [14], may be directly or indirectly targeted by amylin to influence hedonic aspects of eating such as reward-guided behaviors that may contribute to the food selection [15,16]. This review will briefly summarize amylin physiology and pharmacology and then focus on the amylin's role in food reward and the effects of amylin analogs in pre-clinical testing for anti-obesity drugs. We have also included some previously unpublished, original data, because we believe that these data are important to introduce and emphasize certain aspects covered in this review article. These points are important because they have not been covered in other, recently published review articles on amylin. Experimental details in respect to these unpublished data are provided in the Supplementary part of this review.

2. AMYLIN RECEPTOR STRUCTURE AND FUNCTION

The amylin receptor consists of a heterodimer of the calcitonin receptor (CTR) core protein combined with one or several receptor activity modifying proteins (RAMPs) to yield specific amylin receptors [8,17—19]. Two splice variants of the CTR and three RAMPs are known, resulting in at least 6 different subtypes of amylin receptors. Recent data from the caudal hindbrain indicate that individual neurons may in fact express more than a single RAMP, theoretically increasing the number of possible amylin receptor subtypes per cell [20]. Amylin receptor components are widely distributed throughout the central

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nervous system, and a high density of both the CTR and RAMPs is found in the AP of the caudal hindbrain, other circumventricular organs (subfornical organ), the hypothalamus (ARC, VMN) and other brain areas (VTA, LDTg, nucleus accumbens [NAc]) [21–24]. So far, the co-expression of the CTR and RAMPs in single neurons of native tissue has only been shown in the AP of the caudal hindbrain [20], but our work has also shown that non-neuronal cells, in particular microglia, also seem to mediate amylin's effects [10,25,26].

Study of amylin receptor function is complicated by the fact that antagonists that specifically block certain subtypes of amylin receptors are not available [8,27], and prototypical amylin receptors (in particular the amylin-1 receptor resulting from the combination of CTR and RAMP1) also seem to mediate the effects of the related peptide calcitonin gene-related peptide (CGRP) [27,28]. Hence, we currently have no clear picture of the role of specific amylin receptor subtypes for certain amylin functions, or of the importance of the expression of more than one RAMP in single cells.

Upon amylin receptor activation, various intracellular signaling systems are activated. Specifically, amylin increases the expression of cyclic GMP (cGMP) in activated AP neurons [29] and leads to a phosphorylation of ERK [30]. In both cases, there is evidence for a functional relevance of these systems in amylin's effect to reduce eating. On the other hand, transfected cell system studies show that amylin signaling also involves cAMP, intracellular Ca^{2+}, and beta-arrestin [17,22]. But none of these has been linked to specific amylin actions as yet.

3. SITES OF AMYLIN ACTION

3.1. Amylin activation of the brainstem and neuroaxis

The AP is critically implicated in mediating amylin's satiating effect. Local AP administration of amylin decreases eating, while local AP amylin antagonist injection increases it, and blocks the eating inhibitory effect of peripheral amylin [31]. Further, surgical lesion [32] or a specific deletion of noradrenergic AP neurons (see also below; [33]) block the effect of peripheral amylin on eating. In addition, a large array of electrophysiological and imaging experiments provide confirmatory evidence for an important and most likely direct effect of circulating amylin on AP neurons (reviewed in [9,34]).

The amylin-induced activation of AP neurons occurred to a large extent in neurons expressing the noradrenalin synthetizing enzyme, dopamine-beta-hydroxylase (DBH), and presumably the subsequent enhanced release of noradrenaline, possibly in the NTS or the lateral parabrachial nucleus (LPB) [33]. These AP neurons are necessary for peripheral amylin to reduce eating, because even a partial chemical lesion of these neurons is sufficient to abolish the eating inhibitory effect of peripheral amylin; this type of lesion had no effect on baseline food intake [33] and therefore circumvents the problem of a surgical destruction of the entire AP, which itself has a negative influence on eating and body weight gain [16,32].

The activation of AP neurons is the first step in the subsequent activation of a neural pathway that projects rostrally to the forebrain and includes the NTS, LPB, and possibly the central amygdala (CeA). Lesions of the respective brain areas (AP, NTS, LPB) abolish amylin's effect on eating and the expression of the neuronal activation marker c-Fos, indicating that activation was absent in brain areas rostral to the lesion, e.g. in the NTS, LPB, and CeA in AP-lesioned rats, or in the CeA in LPB-lesioned rats [9,35,36]. Indeed using anterograde and retrograde tracing, the AP was shown to project to the NTS, LPB, CeA, and the bed nucleus of the stria terminalis [37].

Furthermore, a recent study suggested that glutamatergic neurotransmission in the AP seems to play a role in mediating amylin effects on eating, and that the amylin receptors appear to be located mainly on presynaptic glutamatergic terminals synapsing with AP neurons [38]; interestingly, our own studies also showed a close apposition of amylin-activated neurons that expressed DBH with VGLUT2-positive boutons [39]. How these effects may be linked mechanistically, and whether this mechanism is physiologically relevant, is currently unknown.

3.2. Amylin action in other brain areas

As mentioned, amylin binding sites have widespread distribution throughout the brain [21]. Similarly, the expression of all critical amylin receptor components has also been described in many brain areas [23,24], and amylin itself may also be expressed selectively in the lateral hypothalamic area [4], although the contribution of the latter to the physiological control of eating remains largely unknown. Recent experiments focused on how amylin receptors in other brain areas outside the AP mediate the physiological actions of peripheral amylin. Among the most prominent of these is the VTA, where components forming the active amylin receptor complex are expressed and where the peripheral administration of the amylin receptor agonist salmon calcitonin (sCT) reduces eating by activating amylin receptors. Importantly, this effect was blocked by the VTA administration of the amylin antagonist AC187 [12,13].

The LDTg has been implicated in processing signals related to the homeostatic but also the hedonic control of eating. It expresses all components of the amylin receptor, and administration of amylin or sCT into the LDTg reduces eating primarily by reducing meal size, similar to amylin's satiating action in the AP [14,40]. Furthermore, administration of the amylin receptor antagonist AC187 into the LDTg reduces the inhibitory effect of peripheral sCT on eating, and depletion of the CTR component leads to increased body weight gain suggesting a physiological relevance of these findings [14].

The relationship between the effects mediated by intra-VTA or intra-LDTg administration of sCT and the previously described AP-mediated effects of amylin and its receptor agonists is unclear at present. Indeed, it is not clear if amylin and its agonists activate several brain areas in parallel. With the exception of the circumventricular organs, including the AP, it is also unclear how much peripheral amylin actually reaches these respective receptor populations as a function of transport across the blood brain barrier [41–43]. It needs to be tested whether similarly to leptin [44], amylin crosses the tanycyte layer around the 3rd ventricle in particular to reach hypothalamic areas. Finally, available data do not distinguish between a direct activation of the pertinent receptors by peripheral amylin or sCT or activation elsewhere (possibly in the AP) and mediation of this effect via projection to the VTA or LDTg, both of which may use may CGRP as neurotransmitter interacting with the amylin-1 receptor [27].

4. AMYLIN ACTION ON FOOD REWARD

Conceptually, controls of eating have often been classified as homeostatic, acting via the caudal hindbrain, including the AP, NTS and LPB, and the hypothalamus, and hedonic, acting via reward pathways in the brain (e.g. [45–48]). However, this distinction is probably oversimplistic since hedonic controls of eating through reward pathways interact with homeostatic control pathways and often can rely on the same signaling molecules. For example, gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1) not only act as homeostatic signals that are involved in the control of meal size or body weight but also influence reward–based aspects of ingestive behavior [49,50]. Food reward is often divided into two components: "liking" and

