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Brainstem mechanisms of amylin-induced anorexia

Catarina Soares Potes *, Thomas Alexander Lutz

Institute of Veterinary Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, 8057 Zurich Switzerland

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ABSTRACT

Amylin is secreted by pancreatic beta-cells and is believed to be a physiological signal of satiation. Amylin's effect on eating has been shown to be mediated via a direct action at the area postrema (AP) via amylin receptors that are heterodimers of the calcitonin receptor core protein with a receptor activity modifying protein. Peripheral amylin leads to accumulation of cyclic guanosine monophosphate, phosphorylated extracellular-signal regulated kinase 1/2 and c-Fos protein in AP neurons. The particular amylin-activated AP neurons mediating its anorexigenic action seem to be noradrenergic. The central pathways mediating amylin's effects have been characterized by lesioning and tracing studies, identifying important connections from the AP to the nucleus of the solitary tract and lateral parabrachial nucleus. Amylin was shown to interact, probably at the brainstem, with other signals involved in the short term control of food intake, namely cholecystokinin, glucagon-like peptide 1 and peptide YY. Amylin also interacts with the adiposity signal leptin; this interaction, which is thought to involve the hypothalamus, may have important implications for the development of new and improved hormonal obesity treatments.
In conclusion, amylin actions on food intake seem to reside primarily within the brainstem, and the associated mechanisms are starting to be unraveled.

The paper represents an invited review by a symposium, award winner or keynote speaker at the Society for the Study of Ingestive Behavior [SSIB] Annual Meeting in Portland, July 2009.

1. Introduction

1.1. Characterization of amylin's effect on eating

Amylin, also known as islet amyloid polypeptide (IAPP), is a 37 amino acid peptide from the calcitonin gene related peptide family [1]. Amylin is co-secreted with insulin by pancreatic β-cells in response to nutrient ingestion at a ratio of about 1:100 (amylin: insulin) [2,3]. Amylin has a short half-life of about 15 min in blood circulation [4].

In rats, food intake (e.g. a 5 g test meal given to overnight fasted rats) results in a rapid increase in the endogenous plasma amylin concentration from a fasting level of approximately 3 pmol/l to postprandial levels of about 15–20 pmol/l measured in aortal blood [5]. This increase in plasma amylin was shown to correlate with the size of the respective meal [5]. Acute intraperitoneal (IP) injections of 1–100 μg/kg amylin decrease eating in rats within a few minutes, and dose-dependently reduce meal size without producing signs of visceral illness [6–8]. Intravenous (3–4 h: 600–2000 pmol kg^{-1} min^{-1}) and intra-area postrema (30 μg over 3 h) administration of the amylin receptor antagonist AC187, at doses that block the eating-inhibitory effect of exogenous amylin, stimulates eating by increasing meal size [9,10]. The lowest dose of exogenous amylin able to produce a significant reduction in feeding yields plasma amylin levels which are only slightly higher than the concentrations measured postprandially [5,11]. For these and other reasons, it is generally believed that amylin fulfils the criteria of a physiological signal of satiation [12,13]. This action seems to be primarily mediated by the area postrema (AP) in the hindbrain (see Section 2 below).

Chronic amylin administration via minipump (2 μg/kg/h, IP) leads to a sustained reduction in food intake due to a decrease in average meal size which is not compensated by an increase in meal frequency [14]. Subsequently, chronic amylin leads to a reduction in body weight gain in rats [7,14]. These findings reported in laboratory rodents have corresponding effects under clinical conditions in humans. The amylin analogue pramlintide (120–240 μg, 3 times/day) causes weight loss in obese subjects starting 2 weeks after the onset of treatment; this weight loss is accompanied by sustained reductions in portion size, in 24 h-food intake, and also in binge eating tendencies [15,16]. Long term treatment for 16 weeks resulted in a marked reduction in body weight that remained significantly lower even 8 weeks after treatment cessation [15].

The role of endogenous amylin in the control of eating has also been investigated using amylin knockout (KO) mice. Most studies using these mice showed no difference in adult (≥4 months) body weight compared with wildtype (WT) animals [17–19]. However, amylin KO

* Corresponding author. Institute of Veterinary Physiology, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 260, CH 8057 Zurich, Switzerland. Tel.: +41 44 635 8813; fax: +41 44 635 8932.
E-mail address: cpotes@vetphys.uzh.ch (C.S. Potes).

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mice showed a higher rate of body weight gain than WT controls, between one and about 4 months of age [18,20]. Average food intake in amylin KO mice was shown to be slightly though not significantly higher than in WT animals during this time window, but food intake was only measured on specific days (about once weekly), and not throughout the entire period from weaning to 4 months of age [19,21].

Rats transgenic for the overexpression of human amylin had a slightly lower body weight than WT controls [22]. This is in principle consistent with the reported effects of exogenous amylin on eating and body weight gain, but food intake in these rats was not measured systematically.

1.2. Other effects of amylin

Amylin seems to exert additional effects at physiological plasma concentrations, such as inhibition of gastric emptying, glucagon secretion, and of gastric acid and digestive enzyme secretion [12,21]. The former two effects are the basis for the use of pramlintide in co-therapy with insulin in diabetic humans because it improves blood glucose profiles in type 1 and type 2 diabetes mellitus patients [23,24]. From the above mentioned actions, the AP was shown to mediate amylin's action to inhibit gastric emptying, eventually involving vagal efferents [25,26]. The site(s) of action for the other effects still need further investigation.

Both acute and chronic administration of amylin and of the amylin receptor agonist salmon calcitonin (sCT) increase energy expenditure, as assessed by indirect calorimetry [7,27–30]. For a review on the effects of exogenous amylin on energy expenditure see [31]. The physiological role of amylin's effect on energy expenditure and the exact site of action are still unclear. One of our own unpublished studies suggests that this effect may also be mediated by the AP because direct low dose amylin or sCT infusions into the AP increased energy expenditure and body temperature, but these findings still require confirmation. Several lines of evidence indicate that amylin may also share characteristics of adiposity signals, such as leptin or insulin. This aspect of amylin action is extensively covered in a recent review [32]. It is not clear whether the same hindbrain neuromechanisms involved in amylin's satiating effect are involved in that latter aspect of amylin action.

2. Amylin site of action at the brainstem

The AP is one of several brain regions that show strong amylin binding in amylin receptor autoradiography studies [33]. The anorectic effect of amylin seems to be mediated by a direct humoral action on neurons in the AP [14,34] which lacks a functional blood brain barrier [35]. Here, we list the available structural and functional data indicating that this activation of the AP is necessary and sufficient to bring about amylin's satiating effect.

The amylin receptor, which is expressed in the AP, is a heterodimer of the type a or type b calcitonin receptor (CTR) as a core receptor and one of the known receptor activity modifying proteins (RAMP), typically RAMP 1 or 3. RAMPs confer specificity of the CTR to amylin [36,37], i.e. they alter CTR pharmacology from calcitonin-preferring to amylin-preferring receptors. The RAMPs are believed to regulate the transport of the core receptors to the cell surface and their glycosylation state, which determines ligand specificity [38,39]. The CTR is densely expressed in the AP [40], and RAMP1 and RAMP3 mRNA have also been discovered in the mouse AP [41]. Amylin-induced c-Fos mRNA, CTR(a) and RAMP3 mRNA expression co-localize in the rat AP [42]; further, most AP neurons in which systemic amylin specifically induces cyclic guanosine monophosphate (cGMP) formation carry the CTR ([40,43] and unpublished observation). Despite the clear evidence that all structural components of functional amylin receptors are present in the AP, one critical experiment still needs to be performed, i.e. an experiment that tests whether the CTR and RAMP1 or RAMP3 co-localize in the same amylin-sensitive AP neurons.

These structural data are complemented by independent functional data. The acute eating-inhibitory effect of amylin was not blocked by either specific hepatic branch vagotomy, by total subdiaphragmatic vagotomy or by capsaicin-induced lesions of peripheral neural afferents that project to the brain [8,44,45]. However, the effect of both acute and chronic amylin administration was abolished in AP lesioned rats [14,34]. Further, local injection of small doses of amylin into the AP inhibited eating by reducing meal size, and AP injection of the amylin receptor antagonist AC187 not only had the opposite effect when given alone, but also blocked the anorectic effect of exogenous peripheral amylin [10].

The behavioral data are consistent with immunohistochemical and electrophysiological studies, in which amylin exerted direct excitatory effects in the AP [43,46]. It is believed that the AP also mediates amylin's action to inhibit gastric emptying, which may require vagal efferents [25,26,47].

2.1. AP neurons are also sensitive to nutrients

Electrophysiological studies have shown that amylin and glucose co-activate AP neurons [48]. This seems to have some functional correlate because amylin's effect to reduce the rate of gastric emptying is not present under hypoglycemic conditions. This phenomenon was considered a fail-safe mechanism to allow appropriate supply of nutrients at a time when they are urgently needed [49]. It is unknown at present if the anorectic effect of amylin is also affected by hypoglycemia in the same way, i.e. whether amylin reduces eating under euglycemic and hyperglycemic, but not under hypoglycemic conditions.

Recent findings indicate that the action of amylin on AP neurons may be affected by protein. A low dose of peripheral amylin (5 μg/kg) induced a strong c-Fos expression in the AP and NTS of 24 h-fasted rats, but not in rats fed ad libitum [50]. Similar to fasted rats, the same amylin dose also induced a strong c-Fos response in rats that received a nutrient-deficient non-caloric mash (NCM) for 24 h before injection. Because selective supplementation of NCM with protein, but not with glucose or fat (lard), attenuated the amylin-induced c-Fos expression observed in NCM fed rats [50], we concluded that protein – or perhaps single amino acids – were responsible to attenuate the amylin-induced c-Fos response in the AP. Consistent with this view, intraperitoneal injection of an amino acid mixture also significantly attenuated the amylin-induced c-Fos expression in the AP in fasted rats [51]. Finally, amylin's anorectic action was stronger in rats fed a 1% protein diet (1% weight/weight) compared to its action in rats fed an isocaloric diet with higher protein percentage (8–18%) [51]. The exact mechanisms that are responsible for these phenomena still need to be investigated. It needs to be tested whether specific amino acids reduce the effect of amylin to activate AP neurons or whether the effect is indirect, e.g. by a protein-induced specific release of some hormone that in part counteracts amylin action in the AP. Our findings indicate that diet derived protein (and amino acids) attenuates amylin's anorectic action and that this depends on the amount of protein itself rather than the caloric intake. The functional implications of these findings, and in particular their clinical relevance for the use of the amylin analogue pramlintide in the treatment of obesity, need to be defined.

3. Brainstem intracellular mechanisms mediating amylin signaling

Numerous studies investigated the central mechanisms that may mediate amylin's effect on eating. Here, we review specific intracellular signaling molecules in neurons of discrete brain areas that are triggered by the peripheral administration of amylin. These molecules will be briefly described in this section, as well as the potential functional relevance in respect to amylin's effect on eating.

Most studies mapping the brain areas activated by amylin made use of the expression pattern of the immediate early gene product c-Fos by immunohistochemistry. Such studies were extremely useful