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Review Article

Role of Amylin in Obesity

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Abstract

Overweight (body mass index, BMI ⩾ 25 kg/m^2) and obesity (BMI ⩾ 30 kg/m^2) are most common nutritional disease affecting more than half of the population in world. The food intake and body weight is regulated by a complex neural network comprising the hypothalamus and the hindbrain. Obesity leads to rise in adiposity signals like insulin and leptin, which leads to insulin and leptin resistance. Increased levels of adiposity and adiposity signals modulate the release and sensitivity to various gut hormones like glucagon like peptide-1, cholecystokinin and amylin which are involved in food intake regulation. Although amylin has multiple actions, including inhibiting secretion of glucagon and insulin, as well as that of lipase and amylase, this review focuses on the relationship between amylin, specific role in central network of appetite regulation and how obesity or a high fat diet affect central signaling of adipose and gut derived hormones.

Keywords: Obesity, adiposity signals, insulin, leptin, amylin

Introduction

Overweight (body mass index, BMI ⩾ 25 kg/m^2) and obesity (BMI ⩾ 30 kg/m^2) are most common nutritional disease in the United States, affecting more than half of the population including 59.4% men and 50.7% for women and overall 54.9% [1]. The food intake and body weight is regulated by a complex neural network comprising the hypothalamus and the hindbrain and it is also thought to be affected by reward system present in the nucleus accumbens and the ventral tegmental area of brain which communicates with each other and receive and integrate neural and humoral information generated in the gut and other peripheral organs reflecting the energy status. Obesity leads to rise in adiposity signals like insulin and leptin, which leads to insulin and leptin resistance. Increased levels of adiposity and adiposity signals modulate the release and sensitivity to various gut hormones like glucagon like peptide-1 (GLP-1), cholecystokinin (CCK) and amylin which are involved in food intake regulation. Obesity or high food intake affect the central nervous system function and leads to the insensitivity to adiposity signals which prevent excess fat accumulation [2]. More knowledge about the function of amylin, a peptide that is elevated in obese persons, may lead to better treatment of obesity.

History of Amylin

Early in the 20th century, two independent researchers described hyalinosis of the pancreas in patients with diabetes mellitus. Amyloid deposits were observed, with the major protein component isolated much later in 1987 and identified as the peptide amylin [3-6]. Many studies have been done on the role of amylin in the development of insulin resistance and diabetes [7]. Amylin has been experimentally administered in diabetic patients to improve glycemic control [8-9]. Other studies of amylin in animals and humans have shown both a direct inhibitory effect on food intake and an indirect effect by slowing gastric emptying [10- 11].

Composition and Synthesis of Amylin

Amylin also known as Islet Amyloid Polypeptide (IAPP) is a 37 residue peptide hormone [12]. It is secreted simultaneously with insulin from the pancreatic β-cells in the ratio of approximately 100:1. IAPP is processed from an 89 residue coding sequence. Proislet Amyloid Polypeptide (Proamylin, Amyloid Polypeptide Precursor, Proislet Protein) is produced in the pancreatic β-cells as a 67 amino acid pro peptide and undergoes post translational modifications and protease cleavage to produce amylin as shown in Figure 1 [13].

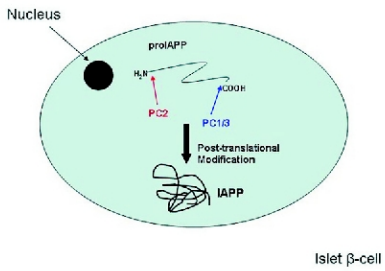

Figure 1. Post-translational Modification of proIAPP to form IAPP

ProIAPP consists of 67 amino acids, which follow a 22 amino acid signal peptide which is rapidly cleaved after translation of the 89 amino acid coding sequence. After released from the signal peptide, it undergoes additional proteolysis and posttranslational to form active IAPP. The amidated C terminus and the disulfide bridge are essential for biological activity of amylin [14].

Receptors

There are three distinct receptor complexes that bind with high affinity to amylin. All three complexes contain the calcitonin at the core and one of three receptor activity modifying proteins, RAMP1, RAMP2 or RAMP3 [15]. A synthetic analog of human amylin with proline substitutions in positions 25, 26 and 29 or pramlintide was approved for adult use in patients with both diabetes mellitus type 1 and diabetes mellitus type 2. Insulin and pramlintide injected separately before a meal controls the postprandial rise in glucose level [16]. Amylin is degraded by insulin degrading enzyme [17]. Amylin is a product of β cells and is simultaneously released with insulin in a molar ratio of 1 to 100 in healthy normal subjects in response to nutrient stimuli like carbohydrate and protein containing meals [18- 19]. Residence time of amylin in the plasma is longer than insulin and it is similar to C-peptide although clearance rate is faster than insulin by the kidneys [20-21]. Because body weight and adiposity influence body kinetic parameters and insulin and amylin

levels, the molar ratio of amylin to insulin is important indicator of relative amylin deficiency [19, 22]. A region between amino acids 20 and 29 is susceptive for amyloid formation in humans and cats [23-24]. In other animals, amylin do not form amyloid even with an amyloidogenic sequence as seen in the dog, hence dog does not develop type-II diabetes [23, 25-26]. Concentrations of amylin in plasma are about 5 to 30 pM/L in normal subjects, 3.4±0.7 pM in lean subjects and 4.7±0.9 pM in obese subjects with normal glucose tolerance. For obese patients with impaired glucose tolerance amylin values of 4.0±0.3 pM were observed and in type 2 diabetes patients 3.7±1.1 pM values were reported [22, 27]. In obese subjects, whether they have impaired glucose tolerance or not the oral glucose tolerance test shows higher amylin response. In addition, in women during pregnancy or gestational diabetes and obese patients higher amylin response to glucose has been observed and no response was seen in patients with insulin deficiency due to type I or type II diabetes [28].

Amylin and Gastric Emptying

The rate of gastric emptying plays a major role in blood glucose homeostasis in normal subjects by controlling the delivery of carbohydrate to the small intestine. Many peptides known to be secreted in response to ingested carbohydrate and amylin and glucagon-like peptide-1 have been reported to inhibit gastric emptying at physiological concentrations [29-31]. Subcutaneous injection of amylin slow gastric emptying in both diabetic and control rats, in greater magnitude than other gut peptides [32]. The rate of gastric emptying of carbohydrate containing liquids is regulated at 2 kcal/min as feedback process from mucosal receptors in the small intestine [33]. The rate of gastric emptying accounts for 34% of the variance in peak plasma glucose after a 75g oral glucose load in normal subjects [34]. The benefit of ingesting soluble fiber on glycemic control in type 2 diabetes reflects retardation of gastric emptying and slower intestinal carbohydrate absorption [35]. Food accumulation in the stomach and gastric distension, amylin may result in earlier meal termination by slowing gastric emptying. The presence of specific binding sites for amylin has been reported in the stomach fundus [36]. Brain regions such as dorsal vagal complex of the brainstem, composed of the nucleus tractus solitarius, dorsal motor nucleus of the vagus and area postrema regulates gastric motility. These regions receive information from visceral afferents and integrate the information to regulate efferent nerve activity to the stomach [37]. Amylin like receptors has been found in two locations in the hindbrain of the rat, the area postrema and the nucleus accumbens [38]. Amylin not inhibited gastric emptying after sub diaphragmatic vagotomy in rats or after surgical ablation of the area postrema [39-40]. Gastric emptying became accelerated in amylin deficient BB rats and amylin antagonists treated rats [41-43]. In a randomized, double-blind placebo-controlled crossover study, delayed gastric emptying of both solid and liquid meals after infusion of pramlintide was reported in men with type 1 diabetes with amylin deficiency [44]. A similar effect was observed in early type 2 diabetic subjects, who were relatively hyperamylinemic and in normal healthy subjects [45-46] In support of amylin's effect on gastric emptying, infusion of pramlintide (50 μg/h) had no effect on plasma glucose when glucose was infused intravenously rather than given orally [47]. Amylin not slowed gastric emptying during hypoglycemia, induced by exogenous insulin. Thus, the feedback mechanism which beneficially restricts nutrient availability by slowing gastric emptying during normoglycemia and hyperglycemia is appropriately blocked during hypoglycemia [48].

Effects of Amylin on Food Intake

Meal termination and satiety may be partly due to the release of gastrointestinal peptides and pancreatic hormones [49-50]. Preclinical data with amylin and clinical data with pramlintide (a human amylin analogue) support a role for amylin in satiety. Pramlintide administration led to sustained weight loss when given for up to one year to type 1 and type 2 diabetic patients at doses close to non-diabetic humans [51–53]. The weight change in type 1 was -0.5 kg for the 30/60 μg pramlintide four times daily and in type 2, -1.5 kg for the 150 μg pramlintide three times daily as compared with +1.0 kg for the placebo group. Amylin diminished insulin induced feeding in mice without affecting the insulin induced hypoglycemia. Amylin injection inhibited food intake in both foods deprived and non food deprived mice in normal or diabetic [54]. Amylin reduced food intake in genetically obese (ob/ob) or lean (ob/c) mice or to diabetic obese (db/db) and lean (db/c) mice at age range of 4 to 22 months [55]. Amylin central bolus infusion (100 pmoles into the third ventricle) significantly decreased 24-hour food intake in 30% rats and the effect persisted over the subsequent week after discontinuation of amylin, without compensation in food intake. Body weight and retroperitoneal fat-pad weight were significantly reduced in the amylin-treated rats [56]. Amylin antagonist AC 187 administration to rats increased ~30% food intake and total body fat but not body weight [57-58]. Activation or inhibition of area postrema, a circumventricular organ outside the blood brain barrier by amylin and its antagonist (AC 187) produce or inhibit anorexia respectively. The stimulatory effect is apparently mediated through formation of second messenger cGMP [59]. Recently, the role of serotonergic, histaminergic and dopaminergic systems was reported. Amylin may induce anorexia through its effect on brain serotonin by increasing the transport of the precursor tryptophan into the brain to inhibit feeding by serotonin action in the paraventricular nucleus. Serotonin reduces the size and duration of meals but does not affect the latency to feed or meal frequency, suggesting increased satiation rather than reduced hunger in rats [60-61]. Amylin's anorectic effect is through stimulating histamine H1 receptors and not by enhancing endogenous histamine release, as indicated by the anorectic effect being absent in mice lacking functional H1 receptors [62]. Additionally, the anorectic effect of amylin was attenuated in rats treated with dopamine D2 receptor antagonists [63]. Amylin also inhibits stimulation of feeding by the potent hypothalamic neuropeptide Y (NPY). When male Sprague Dawley rats received 1nmol of NPY through an intracerebroventricular cannula, subsequent dosing with amylin resulted in dose dependent inhibition of NPY induced feeding. Furthermore when rats received daily doses of 0.5nmol of amylin, 30 minutes before the dark phase, for 6 days, food intake and ultimately body weight were significantly reduced. These rats lost 17.3 ± 6.1 g, whereas their control counterparts gained 7.7 ± 5.1 g. In spite of the reduced food intake, NPY was not elevated suggesting that amylin may regulate NPY production or release [64]. A number of gastrointestinal peptides reduce food intake by stimulating ascending vagal fibers. Whereas truncal vagotomy blocks inhibition of food intake by cholecystokinin (CCK), somatostatin, and glucagon, it does not block inhibition by amylin [55]. One mechanism by which amylin appears to reduce food intake is by augmenting the actions of other peptides such as CCK, glucagon, and bombesin, all of which also increase amylin secretion. However, the CCK antagonist L-364718 did not attenuate amylin's reduction of food intake, suggesting that amylin does not produce its effect through the release of CCK [55]. Instead it appears to be the converse that the anorectic effects of CCK and bombesin depend partly on the presence of amylin or its near cousin, the calcitonin gene related peptide (CGRP) [64]. Amylin is ~50% homologous to the 37-amino acid neuropeptide α and β CGRP, which all act on a family of related G protein-coupled receptors. Both CGRP and amylin peptides have nearly identical N- and C-terminal regions and the disulfide bridge between amino acids 2 and 7 [65]. In contrast to amylin, which is only expressed by the β cell of the pancreas, CGRP is expressed in many tissues, such as the brain, spinal cord, thyroidal C cells and pancreatic islets, and is a potent vasodilator, involved in regulating

blood flow [66]. When amylin action is blocked with a CGRP receptor antagonist, the anorectic effects of CCK and bombesin are also attenuated in rats [64]. There is evidence to support a role for endogenous amylin in regulating body weight and food intake. Combined amylin and CCK, each at sub-threshold doses, is twenty fold more potent in inhibiting food intake in rodents than when administered separately [67]. Both amylin and CCK are naturally secreted in response to mixed meals. Furthermore, there is a 23% to 29% increase in body weight in amylin gene knockout mice [57]. Food intake suppression effect of amylin is not noxious like lithium chloride. Amylin may peripherally produce anorexia by inhibiting nitric oxide, a major regulatory agent in the gastrointestinal tract, because L-arginine, a precursor for nitric oxide, partly reverses the effect of amylin on food intake [68-70].

Amylin and Feeding Behavior

Amylin is co secreted with insulin from pancreatic B-cells. Insulin functions as an adiposity signal and glucagon functions as a satiety signal while amylin has both kinds of signal properties. Like insulin, amylin levels are low during fasting and increase during meals or after glucose administration and relates to body fat. Amylin and insulin are normally co secreted in a fixed molecular ratio (insulin to amylin) of between ten and one hundred. Obesity, diabetes mellitus, pancreatic cancer and certain pharmacological interventions all tend to increase the amount of amylin relative to insulin.

Role of Amylin as a Satiety and Adiposity Signal

Plasma amylin levels increases after meal and it is proportional to meal size [71-72]. Administration of amylin before meal dose dependently decreases food intake in animals due to decrease in meal size, without producing a conditioned taste aversion [73-82]. The effect of exogenous amylin on meal pattern is similar to that of CCK. Plasma amylin is thought to function as a satiety signal by accessing receptors in the area postrema (AP) in the hindbrain having permeable blood brain barrier. Some amount of plasma amylin may also enter the brain via facilitated transport through the blood brain barrier [83]. Administration of low dose amylin directly into the lateral or third cerebral ventricle produces anorectic effect while the blockade of amylin receptors increases food intake and body weight. Anorectic action of amylin is associated with formation of cGMP in the AP [82, 84]. Amylin infusion in the abdominal cavity produces prolonged reduction in food intake and body weight gain which abolished in with AP/NTS lesions animals and rapid rate of weight gain was observed in amylin deficient mice [76, 85-87]. Obese Zucker fa/fa rats have dysfunctional leptin receptors and they become hyperinsulinemic, hyperleptinemic and hyperamylinemic. Administration of amylin antagonist to these rats results in increased food intake due to blockade of amylin receptors [88]. Hence, amylin function as an important adiposity signal since they are insensitive to catabolic action of insulin or leptin. Amylin also reduces meal size in rats on a high fat diet in diet induced obesity model [89-90].

Disruption of Amylin Signaling

Administration of amylin antagonists such as amylin8-37, AC 253 and AC 187 into the AP before meal reduces the anorectic action of exogenous amylin and increases food intake when administered alone [78, 88, 91-92]. It suggests the role of endogenous amylin in the regulation of food intake. Amylin deficient mice showed increased food intake and increased rate of body weight gain compared to normal [86-87].

Interactions of Amylin with other Signals

Report showed that amylin and CCK interact synergistically to decrease meal size [93]. CCK does not produced anorectic effect in amylin deficient mice [94]. CCK antagonists do not affect the anorectic action of amylin while amylin antagonists affect CCK's anorectic action [95]. These results show that endogenous amylin has a neuromodulatory function within the area postrema/nucleus tractus solitarius region that augment other satiety signals same as CCK to the NTS via afferent vagal nerves [96]. Insulin and leptin acts simultaneously in the hypothalamus to affect the brain's sensitivity to meal generated signals. As amylin and glucagonlikepeptide-1 (GLP-1) amylin and glucose activate the same neurons in the AP and AP neurons are also responsive to CCK [77, 97]. AP neurons are therefore able to integrate several metabolic and hormonal signals important in the control of energy homeostasis. Amylin may produce anorexia during pancreatic neoplastic diseases characterized by higher plasma amylin levels [98]. Amylin deficiency may also cause hyperphagia that occurs in IDDM. Long term treatment of type 2 diabetics, overweight and insulin resistant patients with amylin analogue with insulin produces greater weight loss than patients receiving insulin only. Thus, co administration of insulin with amylin might help to reduce weight gain that occurs in type 2 diabetics treated with insulin and insulin analogues [99]. Amylin reduces meal size by stimulating neurons in the AP and amylin signal interacts with other signals controlling energy balance.

Neural System Stimulation by Amylin

Amylin main site of action is AP neurons in the brain. Anorectic action of amylin is abolished in lesioned AP/NTS region [100]. In vitro test, amylin stimulates AP neurons circulating plasma concentrations. Amylin induces FOS expression in the AP, and amylin antagonists Plasma amylin levels increases after meal and it is proportional to meal size [71-72]. Administration of amylin before meal dose dependently decreases food intake in animals due to decrease in meal size, without producing a conditioned taste aversion [73-82]. The effect of exogenous amylin on meal pattern is similar to that of CCK. Plasma amylin is thought to function as a satiety signal by accessing receptors in the area postrema (AP) in the hindbrain having permeable blood brain barrier. Some amount of plasma amylin may also enter the brain via facilitated transport through the blood brain barrier [83]. Administration of low dose amylin directly into the lateral or third cerebral ventricle produces anorectic effect while the blockade of amylin receptors increases food intake and body weight. Anorectic action of amylin is associated with formation of cGMP in the AP [82, 84]. Amylin infusion in the abdominal cavity produces prolonged reduction in food intake and body weight gain which abolished in with AP/NTS lesions animals and rapid rate of weight gain was observed in amylin deficient mice [76, 85-87]. Obese Zucker fa/fa rats have dysfunctional leptin receptors and they become hyperinsulinemic, hyperleptinemic and hyperamylinemic. Administration of amylin antagonist to these rats results in increased food intake due to blockade of amylin receptors [88]. Hence, amylin function as an important adiposity signal since they are insensitive to catabolic action of insulin or leptin. Amylin also reduces meal size in rats on a high fat diet in diet induced obesity model [89-90].block FOS response in the AP. FOS level increases in the AP/NTS after food consumption in rats [101]. The AP/NTS integrate signals related to meals and amylin imparts the region more sensitive to other metabolic signals that reduce food intake. There is no unique amylin receptor gene and the functional amylin receptor in the AP utilizes a calcitonin receptor (CTR) whose amylin specificity and affinity come through the co expression of receptor activity modifying proteins (RAMPs) [102]. The prototypical amylin receptor results from the interaction of RAMP 1or RAMP 3 with the CTR. RAMP1 and RAMP3mRNA have been co localized with amylin induced Fos mRNA in the rat AP, and amylin sensitive AP neurons also express CTR [103]. Amylin produces Fos response in the lateral parabrachial nucleus (LPBN), the central nucleus of the amygdala (CeNA) and the bed nucleus of the stria terminalis [101, 104]. The NTS and the lPBN are relay stations for satiety and other signals to reach forebrain areas. There is no evidence that peripheral amylin has a direct effect on the hypothalam-

us and peripheral amylin or its agonist salmon calcitonin down regulates the expression of orexin and melanin concentrating hormone (MCH) in the lateral hypothalamus (LHA). LHA neurons expressing orexin and MCH are also inhibited by signals coming from insulin and leptin. The LHA is devoid of amylin binding sites hence it might be possible that LHA receives inhibitory input from amylin activated neurons [105].

Pathophysiology of Amylin's Anorectic Action

Amylin has been suggested to contribute to the anorexia occurring during certain pancreatic neoplastic diseases that are characterized by supraphysiological plasma amylin levels [106- 107]. Lack of amylin may also contribute to the hyperphagia that occurs in IDDM since these individuals also lack amylin. Consistent with this, long-term treatment of late stage type 2 diabetics who are overweight and insulin resistant with an amylin analogue in addition to insulin resulted in far greater weight loss than occurred in diabetics receiving insulin only. Thus, co-administration of insulin plus amylin might help to circumvent the increase in body weight that occurs in type 2 diabetics treated with insulin, insulin secretagogues or insulin sensitizers [99, 108]. To summarize, blood borne amylin reduces meal size by stimulating neurons in the AP. Besides enhancing the action of other satiety signals at the level of the hindbrain, the amylin signal interacts with other signals controlling energy homeostasis at the level of the LHA and probably elsewhere. Finally, there is evidence that amylin functions as an adiposity signal in addition to a satiety signal.

Amylin and Treatment of Obesity

In animal and human studies showed that amylin delays gastric emptying and decreases food intake. Obese subjects exhibit hyperamylinemia and their elevated amylin levels may cause down regulation of amylin receptors. Obese persons also suffer from hyperglycemia and increased corticosteroid secretion both of which provoke amylin secretion in response to a meal and could lead to amylin resistance [109]. Pramlintide treatment as an adjunct to life style modification for 12 months with low dose three times daily or higher dose two times daily regimens helped obese subjects achieve greater initial weight loss and enhanced long term maintenance of weight loss [110]. Amylin administration to obese persons may delay gastric emptying and inhibit food intake and overcome resistance and promote weight loss.

Conclusion

Overweight and obesity are most common nutritional disease in the developing countries which affect more than half of the population in world. The food intake and body weight is regulated by a complex neural network involving hypothalamus and the hindbrain and it is also affected by reward system present in the nucleus accumbens and the ventral tegmental area of brain which communicates to receive and integrate neural and humoral information generated in the gut and other peripheral organs reflecting the energy status. Obesity leads to rise in adiposity signals like insulin and leptin, which leads to insulin and leptin resistance. Increased levels of adiposity and adiposity signals modulate the release and sensitivity to various gut hormones like GLP-1, CCK and amylin which are involved in food intake regulation. Obesity affects the CNS function and leads to the insensitivity to adiposity signals which prevent excess fat accumulation. More information about the function of amylin in obese persons may lead to better treatment of obesity.

Conflicts of interest

The author declares no competing interests.

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