The Islet Amyloid Polypeptide hormone Amylin: Its function, effects and uses in medicine

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Amylin is a 37-residue β-cell pancreatic peptide hormone co-secreted in parallel with insulin in response to eating (Kraemer et al., 2011; Vella et al., 2002). Because of amylin's function in diabetes and obesity, members of the healthcare field such as doctors, nurses and registered dietitians who work with these common conditions would benefit from understanding the hormone's endogenous effects and its use as a medication. Though amylin was discovered much later than insulin, research on the peptide hormone has exploded in the last decade, as evidenced by the quantity of contemporary papers published on the subject. The more research is done, the more is discovered about amylin's numerous roles in metabolism and pathology. Amylin has several different names. It is otherwise known as Islet Amyloid Polypeptide hormone (IAPP). Though the term is interchangeable, IAPP is usually used in European research while amylin is preferred in American work. Pramlintide is the name given to the synthetic version of amylin. It has the the same functions and thus is equivalent to the endogenous hormone. This paper will discuss amylin as a glucoregulator in the postprandial period, its effects on adiposity, energy metabolism, its role in contributing to pathology, as well as clinical use of its synthetic analogue to help manage diabetes mellitus and obesity.

Glucoregulation

Studies have found that amylin affects glucoregulation in the postrprandial period by several mechanisms which, in conjunction, have a significant benefit on glycemic control. These mechanisms include slowing gastric emptying, decreasing postprandial glucagon secretion and increasing satiety.

Gastric Emptying

Slower rates of gastric emptying affect the postprandial (after a meal) rise in blood glucose for the simple reason that sugars enter the bloodstream more slowly. Slower absorption into the body means that blood glucose (BG) levels will have smaller incremental areas under the concentration curve when graphing changes in BG over time (Chase, Lutz, Peneck, Zhang, & Porter, 2009). In other words, blood sugars will not peak as high when gastric emptying rates are slowed. A study done in 2002 by Vella et al. looked into the mechanism of pramlintide in diabetics. Type 1 and 2 diabetics participated because both have low or non-existent levels of endogenous amylin (in advanced states of type 2 DM). Participants were randomly assigned to be given either of two different doses of pramlintide or a placebo before eating meals containing isotopic carbon ^{13}C. As the isotopic meal is metabolized, exhaled CO_2 begins to contain ^{13}C. Isotopic exhaled CO_2 correlates to the digestive state of the food, and this was measured in the study.

Results showed a significant, though not dose-dependent, delay of gastric emptying in participants given pramlintide. This effect was shown to be equal in type 1 and 2 diabetics. A relationship between the rate of gastric emptying and plasma concentrations of pancreatic hormones was also demonstrated. This study found that patients given pramlintide had slower gastric motility which correlated with lower pancreatic hormone concentrations in the blood.

These findings are important to elucidating the physiological mechanisms by which amylin cause changes in the body. These mechanisms are complex and not yet well understood, however two explanations are suggested. The first is an effect on the stomach by gut hormones and the second by central neural control (Lutz, 2011; Turek et al., 2010; Vella et al., 2002).

Because the stomach has no receptors for amylin, hormonal control may be exhibited by affecting the actions of other hormones known to inhibit gastric rate such as cholecystokinin (CCK), leptin and incretins GLP-1 and GIP[36]. The second likely scenario is amylin acting on the central nervous system, a hypothesis supported by several animal studies cited by Vella and coworkers (2002). Rats have been shown to have amylin receptors in an area of their brain which controls the vagus nerve, which in turn controls the pancreas and stomach. In further support of this hypothesis, the effects of amylin cease when the vagus nerve was cut in study animals. It is important to also consider the possibility that amylin may cause physiologic changes through both hormonal and central nervous mechanisms simultaneously.

Glucagon Secretion

Research suggests that amylin's effect on gastric emptying alone can alter postprandial glucose response. However this is only one of IAPP's actions, as the hormone has been shown to also decrease postprandial glucagon secretion.

It is first important to understand glucagon's role in the body. Glucagon is another pancreatic peptide hormone which functions essentially opposite of insulin. It signals the liver to engage in gluconeogenesis and gluconeolysis, typically during times of hypoglycemia to raise blood glucose. Diabetics however, lack the ability to suppress glucagon secretion during hyperglycemic postprandial periods. Meier, Kjems, Veldhuis, Lefebvre, & Butler's study from 2006 indicates that the inappropriate glucagon secretion typical of diabetes is due to a lack of insulin, but the 2009 study from Chase et al. tells us that exogenous insulin does not have the same inhibitory effect as endogenous insulin on pancreatic alpha cell's secretion of glucagon. This means that insulin-treated diabetics will continue inappropriate glucagon secretion into the

postprandial period despite administration of mealtime insulin and will struggle with managing blood glucose. The study from Chase and others found that pramlintide suppresses glucagon after a meal while treatment with exogenous insulin does not. In support of this finding, one clinical investigation gave type 1 diabetics a meal with a known amount of carbohydrate and injected the control group with their normal insulin bolus while the experimental group received insulin plus a dose of pramlintide (Heptulla, Rodriguez, Bomgaars, & Haymond, 2005). Participant's blood concentrations of glucose, glucagon and IAPP/pramlintide were monitored for seven hours afterward. Findings from this trial indicated that pramlintide significantly lowered blood glucose excursions and reduced glucagon concentrations. These results point to IAPP's significance as a glucoregulator by preventing untimely secretion of glucagon, thus preventing or reducing hyperglycemic postprandial events.

Adiposity

In addition to the glycemic control that amylin has been shown to exert, it is believed to be a signal for adiposity; the amount of fat in the body. Experimental evidence indicates a relationship between obesity and amylin, and shows amylin's similarities to other known adiposity signals. Furthermore, amylin's role in satiation contributes to reduced adiposity.

Several discoveries point to a positive relationship between IAPP levels and obesity as evidence for amylin as an adiposity signal (Lutz, 2012; Turek et al., 2010, Woods, 2004). First, plasma amylin concentrations have been shown to be higher in obese rats, and secondly, rats which are fed to become obese also showed higher levels. Lastly, these results were confirmed in obese humans who had significantly higher amylin levels when fasting and postprandial than their thinner peers (Enoki et al., 1992).

More than a correlational relationship, hormonal and genetic interventional experiments show causal relationships between amylin and obesity (Lutz, 2011; Lutz, 2012; Weilinga et al., 2010). Rats who were administered amylin decreased body weight specifically in fat mass. Moreover, when the effects of amylin's adiposity were blocked with an amylin agonist, the mice receiving the agonist medication gained significant fat mass. This finding tells us that without amylin's mediation, adiposity increases. This implies that endogenous plasma amylin found in obese specimens is higher because amylin is not working as effectively (likely due to insensitivity) and thus is overproduced. Further tests investigated whether IAPP's action was substantial in duration. Rats were put into three groups and either overfed, fed normally or underfed, and half from each group were given amylin. Regardless of their previous nutritive states, at the end of two weeks, all amylin-infused rats had lower body weights than their counterparts. This suggests that amylin levels in the blood may set homeostatic weight controls to a lower mass and contribute to weight constancy.

Genetically-induced changes in endogenous amylin levels also provide similar evidence. In 2010, Turek and others created mice whose amylin-encoding gene was knocked out. They found that these deficient mice were heavier than wild-types. This same study also concluded that the body's response to leptin, another known and well-studied adiposity signal, is modulated by amylin and synergistically leads to weight loss.

Satiation

IAPP's action in satiation is intricately related to adiposity (Woods, 2005). Regulating nutrient intake by satiation alters carbohydrate consumption, so satiation is also related to glucoregulation. With that said, satiation is a complicated cascade of signals, so much so that the

gut is often referred to as the second brain. As much of the subject matter is beyond the scope of

this paper, (and partly still beyond the understanding of modern science) the information

presented here is general. Research indicates that amylin reduces eating over a sustained period

by acting in the brain on the area postrema, a structure in the medulla oblongata that controls

feeding.

Amylin is seen to work like a known satiation signal CCK, a gastrointestinal peptide

hormone which has many physiological effects including influences similar to amylin's

mediation of gastric emptying and satiation (Woods, 2004). Amylin administration, unlike CCK,

appears to cause sustained reductions in food intake and body weight without compensatory

behavior such as increased meal frequency (Lutz, 2011). Animal studies have shown that one

injection of IAPP can reduce eating for up to 24 hours. What's more, after that initial reduction

of 24 hours, animals did not "make up" for the deficiency in following days. This data is

suggestive of amylin's ability to cause weight loss and to prevent re-gaining it. In another

experiment, rats were centrally administered amylin (i3v infusion: a direct injection in the third

ventricle of the brain) and monitored for meal size and frequency. Results were similar to

peripheral IV infused amylin, demonstrating significant weight loss, reduced meal sizes and in

higher doses, less frequent meals.

Lutz adds in his 2012 article that amylin lowers food intake in rodents without causing a

conditioned taste aversion. This was tested by giving rats amylin and recording their preferences

between water and sugar-water solutions. There were no changes found in preferences after

amylin administration, though a large total reduction in eating was noted. A control substance

known to cause taste aversion was separately administered and did trigger a significant change in preferences.

The question of how amylin produces satiation is not well answered. It has not yet been discovered whether amylin's signals for adiposity and acute satiation are separately processed in the brain (Lutz, 2011; Lutz, 2012). Ongoing research is looking into the neural pathways by which amylin creates the sensation of satiation. So far, it is suspected that at least part of the pathway is through the release of dopaminergic catecholamines.

Though much of the research presented in this section concerning adiposity has been done on rodents, it is not unreasonable to assume that the results also apply to humans, but perhaps the magnitude of effects vary between species (Enoki et al., 1992; Lutz, 2012). More research on pramlintide use in humans will come later in this paper. Nevertheless, a number of studies have shown that high levels of amylin correlate with obesity and insensitivity is suspected to have a causal relationship with obesity. Furthermore, amylin plays a part in the satiation cascade, secondarily affecting adiposity and glucoregulation by limiting nutrient intake.

Energy Metabolism

A study Lutz published in 2011 suggests that the weight-reducing action of IAPP may be due to an effect on energy expenditure in addition to reducing energy intake by satiation. This paper reviews a number of studies done on rodents where the effect of amylin on energy expenditure was clearly demonstrated. Rodents were pair-fed so energy intakes were the same while only one was given amylin. The amylin-dosed rodent had a significantly lower body weight and adiposity at the end of these studies. IAPP appears to either increase total energy expenditure or prevent the body's natural decrease in energy expenditure when calorie intake is

restricted. These effects are thought to perhaps increase the proportion of metabolically active

lean body mass or by increasing fat oxidation, though experiments have not had consistent

results.

Pathology

The information presented thus far explains metabolic actions of endogenous IAPP as it

is intended to work. Scientists have discovered however, that amylin also has significant

pathologic effects when it starts to work in ways it was not meant (Aitken, Loomes,

Konarkowska, & Cooper, 2003; Mandrup-Poulsen, 2001; P. Westermark, Engstrom, Johnson, G.

Westermark, & Betsholtz, 1990). In the bodies of persons affected by this pathology, misfolded

molecules of IAPP aggregate into insoluble fibril structures that can become quite large. These

aggregations of amylin are called amyloid. Amyloid causes cystic pockets in the tissues where

they occur and it is believed that this may lead to the beta cell dysfunction that causes diabetes.

In fact, the most characteristic morphological alteration, seen in up to 95% of type 2 diabetics, is

the extracellular deposition of amyloid. Interestingly, much research is also being done on

amyloid-forming proteins elsewhere in the body, namely in the brain, and it is suspected that this

same amyloid mechanism is responsible for the occurrence of Alzheimer's disease and

Parkinson's (Baine et al., 2009; Fandrich, 2012). Recent research in this area concentrates on

three things: the mechanisms of aggregation, toxicity, and ways to prevent, reduce or dissolve

amyloid.

Mechanism of Aggregation

In order to stop IAPP from aggregating, scientists must first understand how the protein

comes together in the first place. A study in 1990 by Westermark et. al. noted that only a limited

number of species are prone to amylin amyloidogenesis. Their study investigated how the amino acid sequence in amylin varied across species. Westermark's team synthesized IAPP and substituted amino acids one by one and in groups, and then observed their tendencies to aggregate. By this process of elimination, they found that residues in positions 20-29 were primarily responsible for aggregate action. Further studies have found a secondary amyloidogenic region at residues 13 and 15-17 (Fox et al., 2010). Other analysis adds to this, indicating that assembly is due to hydrophobic interactions and the formation of hydrogen bonds (Cheon et al., 2007; Fandrich, 2012). Using computer models, Cheon and coworkers revealed that oligomeric assembly occurs in two steps. First, assembly is characterized by hydrophobic interactions to sequester hydrophobic residues away from the solvent, resulting in the formation of elastic oligomer groups. In the second step, interchain hydrogen bonding drives reorganization, and can expose hydrophobic residues. This hydrophobic exposure is important, because it can explain amyloid toxicity to membranes and therefore dysfunction of the protein and its cell.

Toxicity

As previously mentioned, amyloid-filled cystic pockets may lead to β-cell dysfunction (Mandrup-Poulsen, 2001), but we have not yet seen how amyloid exerts its toxicity. Amyloid takes different forms as the aggregations grow, and these have different effects. Scientists are still debating which forms are responsible for cell death.

First is the amyloid hypothesis; the mature aggregated fibrils of amyloid proteins, cause cell death. Mature amyloid fibrils are fairly rigid and large. This may cause physical impairments in the tissues, activate inflammatory responses or overload protein degradation machinery.

The second and more conventional oligomeric hypothesis posits that smaller, thinner aggregations in oligomeric intermediate stages cause toxicity (Eliezer, 2006; Fandrich, 2012; Sokolov, et al., 2006). Mechanisms of oligomer-mediated toxicity can be similar to mature fibrils, except for the physical impairment of tissues unique to fibrils. Unique to oligomers on the other hand, is their clearly observed activity in increasing membrane permeability. Amyloid intermediates are thought to form ion channels or affect the integrity of cellular lipid bilayers, breaking the barrier separating the intracellular and extracellular spaces. This increase in ion permeability disrupts homeostasis and leads to cell death. Though oligomers bare similarity to other known toxins that form pores in cell membranes, electrophysiology experimentation from Sokolov and others (2006) favors membrane integrity disruption. Their work displayed increases of membrane permeability without any evidence of ion channel formation. More research concerning the mechanisms of toxicity is important because a better understanding may help target therapeutic development to prevent membrane permeabilization and thus prevent cell death.

Inhibition or Dissolution of Amyloid Aggregations

 IAPP is the most amyloidogenic protein known and research on how to stop or reverse IAPP aggregations has been intensive. A number of compounds have been shown to reduce or eliminate aggregation *in vitro* (Aitken et al., 2003). Aitken et al. investigated the ability of several polycyclic compounds, including the common antibiotic tetracycline, to interfere with the conversion of synthetic human amylin into its insoluble amyloid form. Remarkable inhibition was seen with several of these compounds at a 1:1 molar ratio with amylin. Tetracycline was shown to still hinder conglomeration too, though to less effect compared to other compounds.

Other chemicals such as resveratrol, found in red wine, and dyes like Congo red have been investigated for effective inhibition properties (Evers et al., 2009; Watson, Lander, & Selkoe, 1997). More research is targeted at developing compounds that specifically interact with the protein regions responsible for aggregation through hydrophobic interactions and hydrogen bonding (Amijee, Madine, Middleton, & Doig, 2009). These compounds compete for aggregation regions with other molecules of amylin, ultimately preventing conglomeration.

We have seen that aggregations cause damage by physical impairment and membrane disruption (Mandrup-Poulsen, 2001). Though some compounds being researched offer the promise of preventing aggregations and thus the diseases that occur because of them, they are not yet practical as interventions. To reach high enough concentrations to work would require ingesting large amounts of the chemical and this would have side-effects. Additionally, there are many challenges in directing these compounds to the parts of the body where they need to be. The search for other chemicals with anti-aggregation properties at lower concentrations continues.

Clinical Use

Because of the pharmacodynamics that amylin has displayed in glucoregulation, adiposity and energy metabolism, the use of its synthetic analogue pramlintide in the management of diabetes and weight loss were indicated after safety of its administration was verified in clinical trials. It is also essential to discuss the role of nutrition in disease management, and how the addition of pramlintide can support efforts.

Diabetes

Clinical trials have evaluated the administration of pramlintide to help control diabetes and found it to be effective (Chartrand, 1997; Lush, Darsow, Zhang, Lorenzi, & Frias, 2007; Younk, Mikeladze, & Davis, 2011). Pramlintide was first tested for its ability to improve glycemic control, and found that over the long-term, patients given the drug had sustained reductions in average HbA1c scores indicating improved glycemic control with fewer episodes of hyperglycemia. Adding pramlintide to a treatment regimen also reduced the amount of basal insulin patients required, and caused no weight gain (unlike treatment with exogenous insulin). These results make pramlintide a possible alternate to the addition of mealtime insulin if treatment intensity needs to be increased in order to control BG.

Weight Loss

After considering results from animal studies, clinical trials were conducted with humans to test pramlintide's use as a tool for weight loss (Lutz, 2012). Results were promising, especially when pramlintide was combined with metreleptin, leptin's synthetic analogue (Ravussin et al., 2009). Single hormone treatments were effective, but combination therapy worked to decrease body weight an average of 12% in five months with more potential loss projected. It is suspected that a maintenance dose would need to be continued to sustain weight loss.

Safety

Considering that amylin has been shown to have pathological effects, one may wonder if giving people the hormone would be safe. In fact, the synthetic version has been altered to prevent aggregation, and its administration had been demonstrated to be well tolerated.

As we saw in the study from Westermark and coworkers, several specific amino acids give rise to aggregation (1990). In the synthetic version, these residues are modified or swapped out for others so to no longer have amyloidogenic properties, but maintain its pharmacodynamics (Heptulla et al., 2005; Vella et al., 2002).

Other known risks of pramlintide include nausea, vomiting and increased risk of hypoglycemic events, especially when combined with another hypoglycemic agent such as insulin, most especially acute mealtime insulin (Lush et al., 2007). Careful management of dosing is thus required, though some risk is removed by substituting pramlintide for mealtime insulin rather than adding it. Nauseous responses to drug administration will generally decline after an adjustment period of few weeks.

Pramlintide & Nutrition

In the face of pramlintide's new clinical roles, we must evaluate how this could relate to nutritive therapy which is the primary method of managing type 2 diabetes mellitus and obesity. In both conditions, pramlintide can be used to supplement nutrition therapy in the case that a person cannot reach desired goals with diet alone. With diabetes, the goal is to gain optimal glycemic control by limiting spikes, using carbohydrate controlled meal plans and low glycemic index foods. The hope of nutritional management is to reduce or prolong the need for additional treatment, including pramlintide. A nutritional consultation with a registered dietitian (RD) is recommended to ensure that patients have sufficient nutrient intakes for a healthful diet. If necessary, nutrient deficiencies may be compensated for with supplements or other dietary changes. Considerations for weight loss must also be taken to account. A pramlintide-assisted intake-restricted diet has to be monitored closely to ensure that the patients meet macronutrient

requirements, such as sufficient complete protein, essential fats, and adequate carbohydrates to prevent hypoglycemic episodes. Because RD's frequently work with diabetics and obese patients, it is important for them to be familiar with all treatment options including medications to supplement weight loss efforts. Furthermore, an in-depth understanding of the disease process and the normal functions of the body are paramount to treat disease. For these reasons, the RD and other health professionals should be knowledgable about amylin and pramlintide.

Conclusion

In summary, amylin is an important and physiologically active hormone, which helps with glucoregulation by delaying gastric emptying and suppressing glucagon release. It also appears to raise energy metabolism, and acts as an adiposity and satiation signal. The hormone can become pathological when it aggregates, causing tissue damage, and scientists are still researching ways to prevent this. Pramlintide has been shown to be useful in clinical settings for glycemic regulation in diabetic patients and weight loss. The addition of this medication has positive effects on nutritive therapy. The future is promising, as continued research provides a better understanding of amylin's physiological and pathological effects, and its potential for treating disease.

Acknowledgements

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