Diabetologia (2010) 53:1046–1056
DOI 10.1007/s00125-010-1671-6

FOR DEBATE

Toxic oligomers and islet beta cell death: guilty by association or convicted by circumstantial evidence?

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Received: 24 August 2009 / Accepted: 4 December 2009 / Published online: 25 February 2010

Abstract Type 2 diabetes is a progressive disease characterised by islet amyloid deposits in the majority of patients. Amyloid formation is considered a significant factor in deterioration of islet function and reduction in beta cell mass, and involves aggregation of monomers of the normally soluble beta cell peptide, human islet amyloid polypeptide (hIAPP) into oligomers, fibrils and, ultimately, mature amyloid deposits. Despite extensive in vitro studies, the process of hIAPP aggregation in vivo is poorly understood, though it is widely reported to promote cytotoxicity. Recently, studies have suggested that only the early stages of fibril assembly, and in particular small hIAPP oligomers, are responsible for beta cell cytotoxicity. This challenges the prior concept that newly formed fibrils and/or mature fibrillar amyloid are cytotoxic. Herein, evidence both for and against the toxic hIAPP oligomer hypothesis is presented; from this, it is apparent that what exactly causes beta cell death when hIAPP aggregates remains debatable. Moreover, substantially more work with more specific reagents and techniques than are currently available will be required to identify conclusively the toxic species resulting from hIAPP aggregation. Keeping an open mind on the nature of the cytotoxic insult has implications for therapeutic developments and clinical care in type 2 diabetes.

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Abbreviations

hIAPP Human islet amyloid polypeptide
IAPP Islet amyloid polypeptide

Introduction

Type 2 diabetes is characterised by the hyperglycaemia that results from an interplay of reduced insulin secretion, impaired muscle and adipose tissue insulin action, and increased hepatic glucose output. The reduction in insulin secretion from pancreatic islet beta cells results from the combination of beta cell dysfunction and an absolute deficiency of beta cell mass [1]. Islet amyloidogenesis, which involves aggregation of another secreted beta cell product, islet amyloid polypeptide (IAPP or amylin), has been reported at post-mortem to occur in 40% to 90% of patients with type 2 diabetes [2–6], and extensive amyloidosis has been shown to be associated with reduced beta cell function and/or mass [7, 8]. Whether aggregation of human IAPP (hIAPP) is a cause or consequence of beta cell demise in type 2 diabetes and whether it occurs intra- or extracellularly remains debatable, despite years of investigation. The data addressing many of these issues have been reviewed previously [9, 10] and thus will not be discussed here.

The process of amyloid formation is considered to be similar for hIAPP and the A-beta peptides of Alzheimer's disease. Given that the molecular size and some characteristics of these amyloidogenic peptides in vitro are similar, there have been some direct transpositions to hIAPP from results on the behaviour of A-beta. Recently, literature has emerged suggesting that only oligomers of A-beta [11] or hIAPP [12] induce cell death. This contradicts the prior concept that newly formed fibrils and/or mature fibrils and amyloid deposits are cytotoxic [13, 14]. Herein, we discuss evidence both for and against this toxic oligomer hypothesis for hIAPP and conclude that while oligomers may contribute to hIAPP-mediated cytotoxicity, at the present time there is insufficient evidence to conclusively prove this hypothesis.

Amyloid formation in Alzheimer's disease: a model for amyloid formation in type 2 diabetes?

A number of major diseases exist where it is now thought that protein aggregation and, in particular, cytotoxicity of non-fibrillar aggregates may play a significant role in the disease process (e.g. transthyretin in familial amyloidotic polyneuropathy [15] and α-synuclein in Parkinson's disease [16]). Perhaps the most widely studied of these is Alzheimer's disease.

It has long been appreciated that Alzheimer's disease and type 2 diabetes share many similarities [17]. Both are diseases of ageing that have a genetic predisposition and are linked to pathologies associated with protein aggregation (aggregation of A-beta in the case of Alzheimer's disease). For this reason, the type 2 diabetes field has utilised the more extensive literature on oligomers of A-beta, including information on A-beta toxicity in the brain, to model what might be occurring with hIAPP in the islet. While it is tempting to assume similar mechanisms may explain amyloid-associated cytotoxicity in both diseases, it is important to recognise that a number of noteworthy differences may exist when comparing amyloidosis in Alzheimer's disease and type 2 diabetes. One example is that in Alzheimer's disease, apolipoprotein E has been postulated to promote the deposition and fibrillisation of A-beta [18–20], whereas in type 2 diabetes, apolipoprotein E has been found not to be critical in islet amyloid formation [21].

Studies of A-beta aggregation and toxicity have been more extensive than those of hIAPP and data exist to support the toxic oligomer hypothesis in Alzheimer's disease [11]. Specifically, oligomeric A-beta has been identified and characterised in homogenates of normal and diseased brain [22], with these oligomers being shown to be toxic to cultured neurons [23]. The text box (Comparison of key evidence supporting the toxic oligomer hypothesis of A-beta in Alzheimer's disease and IAPP in type 2 diabetes) summarises the key evidence for the toxic oligomer hypothesis in Alzheimer's disease and indicates how much of this evidence remains to be generated for islet amyloidosis.

Comparison of key evidence supporting the toxic oligomer hypothesis of A-beta in Alzheimer's disease and IAPP in type 2 diabetes

Investigation	Alzheimer's disease		Type 2 diabetes	
	Evidence?	References	Evidence?	References
Detection and quantification of oligomers in human tissue	✓	[22, 75–77]	✓	[40][a]
Characterisation of oligomers from human tissue	✓	[78]	×	-
Extraction of oligomers from human tissue and application to cultured cells to determine cytotoxicity	✓	[78]	×	-
Synthetic peptides capable of forming oligomers do or do not accurately mimic human peptides in vivo	✓	Reviewed in [79]	×	-
Use of cell-derived human oligomers for in vivo experiments	✓	[80]	×	-
Oligomer-binding antibodies can ameliorate neuronal (for A-beta) or islet (for IAPP) cell toxicity	✓	[36, 81]	×	-
Oligomer-specific inhibitors can ameliorate neuronal (for A-beta) or islet (for IAPP) cell toxicity	✓	[82, 83]	×	-

[a]A11-immunoreactivity in paraffin-embedded human pancreas tissue. See related discussion regarding specificity of A11 in main commentary

What is an oligomer?

In simple chemical terms, an oligomer is defined as an assembly of molecules comprising a number of monomeric units. Oligomers represent intermediates in the fibril-formation pathway and are commonly referred to as prefibrillar/soluble aggregates [24, 25]. Figure 1 illustrates the stages in islet amyloid formation during which hIAPP undergoes aggregation, ultimately into a fibrillar state. This conformational change is highly dependent on particular amino-acid sequences; the composition of residues 20–29 of hIAPP is particularly important, although this is not the sole determinant of the ability to form amyloid [26]. The amino-acid sequence of rodent IAPP differs from hIAPP so that it is not predisposed to aggregate into fibrils or form mature amyloid deposits in vivo [26].

The process of fibril formation has been studied extensively in vitro and is consistent with a nucleation-dependent assembly mechanism [27]. When monitored using the thioflavin T assay, the kinetics of assembly typically involve an initial lag phase followed by a sigmoidal transition and finally a steady-state phase (Fig. 1). The lag phase represents the thermodynamically unfavourable nucleation process [28] whereby individual monomers assemble into oligomeric

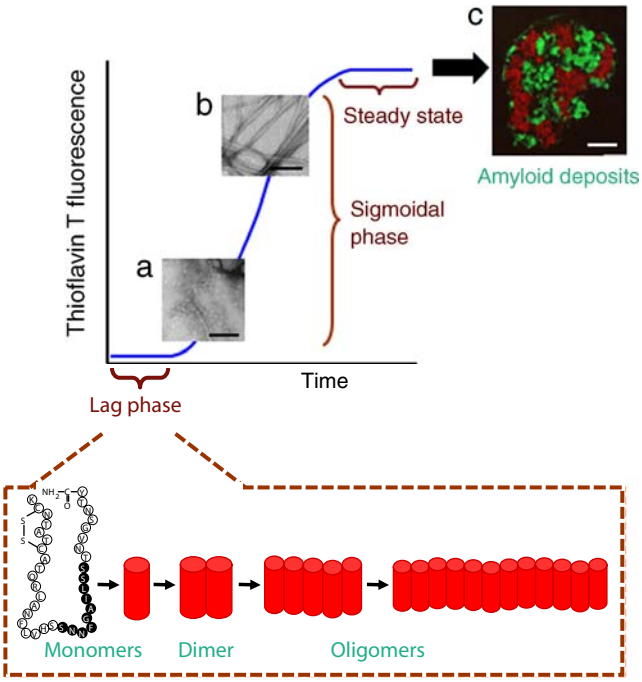

Fig. 1 Stages of islet amyloid formation. Human IAPP is a 37-amino-acid peptide; residues 20–29 (shaded) are particularly important for conversion of the molecule to beta sheet conformation and assembly into fibrils. Fibril formation in vitro can be measured using the thioflavin-T-binding assay, which typically shows a lag phase and a sigmoidal increase to a steady state. This reflects the assembly of fibrils. The exact process of assembly from monomer to fibril in vivo is unknown. A simplified version of events is depicted below the graph. Initially IAPP monomers are in random structure. Off-pathway oligomers may also be formed but are not depicted in the diagram. Under particular conditions the monomer assumes a beta conformation (depicted by the red rod), forms dimers, small oligomers and larger multimers. This occurs during the lag phase of the thioflavin T assay. As the assemblies extend, **a** protofilaments (4 nm diameter, visible by electron microscopy), are formed which themselves assemble into **b** fibrils (two to four protofilaments, 10 nm diameter); these assemblies are thioflavin T positive. Progressive fibril formation leads to **c** islet amyloid deposits (green), which can occupy a large proportion of the islet, replacing insulin-producing cells (insulin is shown in red) in type 2 diabetes. Scale bars: **a**, **b** 100 nm; **c** 50 μm

species. The sigmoidal phase signals individual protofibril and fibril growth and elongation, and the steady state represents a stable equilibrium between fibrillar IAPP and residual monomeric IAPP.

A small number of studies have suggested that hIAPP oligomers may be an 'off-pathway' species [29, 30] and thus are not obligate intermediates for fibril formation. The evidence for this relies on the differential ability of inhibitory compounds to specifically block oligomerisation and/or fibrillisation. These data must be interpreted with caution as it has been demonstrated that some inhibitors may interfere with biophysical assays of amyloid formation rather than with the fibril self-assembly process [31, 32]. In addition, experimental conditions such as peptide concentration, type of solvent, presence of lipids or glycosoaminoglycans, agitation, temperature, pH, ionic strength, spontaneous non-enzymatic modifications and even the mode of sample purification can all influence the propensity of hIAPP to form on- or off-pathway species [29, 30, 33]. It is unclear whether these factors have been carefully considered in studies of potential off-pathway oligomers.

How can one assess the presence of an IAPP oligomer?

High-resolution microscopy techniques, namely electron and atomic-force microscopy, are most often used to detect and measure the dimensions of small amyloidogenic species, including oligomers. Spectroscopic techniques can also be employed, although methods such as nuclear magnetic resonance generally lack the time resolution necessary to obtain a snapshot of the assembly process. Light scattering is the classic biophysical method for following aggregation and self-assembly reactions such as in amyloid formation. Also, cross-linker methods, using either classic chemical or photo-induced cross-linkers, can be used to trap oligomeric species for isolation; however, there is always the risk that cross-linking alters the structure of the oligomeric assembly. Further, analytical ultracentrifugation can be used to deduce the size of oligomers and real-time nano-electrospray ionisation mass spectrometry can be used to identify and monitor individual oligomers within complex mixtures throughout fibril formation. Thus, while a number of methods can be employed to identify oligomers (reviewed in [34]), studies using such approaches for hIAPP are largely lacking. Some interpretations of hIAPP data have been based on observations in A-beta studies, perhaps because A-beta can be more easily followed throughout fibrillogenesis as it aggregates relatively slowly in vitro (hours/days) compared with hIAPP (minutes). Certainly this property of hIAPP considerably limits the utility of classic techniques such as light scattering in studying the time-dependent formation of islet amyloid. Thus, the most reliable and informative techniques for analysis of hIAPP aggregation are scanning tunnelling electron microscopy and atomic-force microscopy, as both produce single-particle two-dimensional profiles. In addition, atomic-force microscopy can provide qualitative and quantitative data, and can be used for time-resolved detection of hIAPP oligomer formation.

What direct evidence is there that hIAPP oligomers exist in vivo?

Conformation-dependent antibodies have been developed and are reported to recognise oligomers but not monomers or fibrils of several amyloidogenic proteins [35, 36]. Polyclonal serum was produced by vaccination

of rabbits with A-beta oligomers that were covalently coupled to colloidal gold in order to stabilise the oligomeric structure. Validation of the resultant antibody, A11, was performed to ensure its specificity and evaluate its reactivity kinetics. It was shown to detect soluble oligomers from many different types of amyloid, including those formed by hIAPP [36]. Interestingly, recent publications document that the A11 antibody also binds natively folded proteins that display anti-aggregation activity, such as heat shock proteins [37], as well as α-synuclein fibrils [38]. These findings call for caution when identifying A11-reactive proteins, as the antibody may not bind exclusively to pathogenic amyloidogenic oligomers. The antibody I11 was produced by vaccination of rabbits with hIAPP oligomers, using a method identical to that used for A11 production. Validation of the specificity of this antibody has not been as thoroughly reported. While I11 reportedly detected oligomeric hIAPP in an ELISA, dot blot experiments did not include the critical hIAPP monomer and fibril controls to prove that it did not cross-react with other species in the hIAPP aggregation pathway [29]. Unexpectedly, investigators interested in hIAPP aggregation in vivo have chosen preferentially to use the A11 antibody in order to detect hIAPP oligomers [39, 40]. This may in part be due to the A11 antibody being commercially available, whereas I11 is not.

When using the A11 antibody to identify oligomers in situ an important consideration is how the tissue was prepared. Despite assurances by commercial sources that the antibody works in paraffin-embedded tissue sections, this may not be the case [12]. As the antibody does not bind an amino-acid sequence but rather a conformational epitope that may be altered by tissue processing, frozen tissue sections are required for the detection of hIAPP oligomers. This said, a recent study of paraffin-embedded human pancreas tissue obtained at autopsy demonstrated A11 immunoreactivity [40]. While the authors state that antibody recognition is of hIAPP oligomers, the actual identity of the target is questionable, given how the tissue was processed. Lin et al. have also performed A11 immunostaining of frozen pancreas from hIAPP transgenic mice and concluded that hIAPP oligomers do exist, but do not co-localise with amyloid fibrils [39]. A significant caveat to such immunostaining studies is that aggregated A-beta [41], A-beta precursor protein and the proteases that generate A11-immunoreactive A-beta [42] have all been reported to exist in pancreas, and A-beta co-localises with IAPP in islet amyloid deposits in type 2 diabetes [41]. Moreover, other proteins known to be recognised by A11, such as heat shock proteins and transthyretin, are also found in pancreas [37]. Thus, it appears the A11 antibody has rather limited utility in detecting hIAPP oligomers in situ, unless it is conclusively demonstrated that all positive staining is due to detection of hIAPP oligomers.

What is the evidence for toxicity of hIAPP oligomers but not amyloid fibrils?

Studies that have employed time-lapse atomic-force microscopy have provided direct evidence for the existence of hIAPP oligomers in vitro, delineating a number of their properties including dimensions, mass, and time dependence of their growth [43]. Based on indirect evidence, a number of other studies have also concluded that hIAPP oligomers exist and are involved in cellular toxicity. First, the failure to detect mature amyloid fibrils in conjunction with cell death has been taken to mean oligomers of hIAPP are the culprit, even if hIAPP oligomers (or even fibrils) were not specifically measured [44]. Second, transgenic rodent models of islet amyloidosis that develop spontaneous diabetes, but without islet amyloid visible by light microscopy, have been shown to exhibit beta cell loss [45, 46]. Third, beta cell loss has been shown to occur at maximal frequency when amyloid deposition is minimal [45, 47], suggesting an intermediate in the fibrillisation pathway could be the toxic species. While this may indeed be the case, the presence of hIAPP oligomers and their association with cell death were not directly assessed in these studies, but were assumed. Moreover, an important consideration when interpreting data derived from transgenic rodent models is that overexpression of hIAPP—exceeding that observed in humans—may itself promote cellular toxicity, probably through endoplasmic reticulum stress [48, 49]. This factor has been considered in some studies where transgenic mice expressing a non-aggregating homologue of hIAPP were used as controls [46]; these mice did not develop beta cell degeneration.

Vaccination studies using the A-beta oligomer have also provided indirect evidence for the toxic oligomer hypothesis. Following vaccination, hIAPP transgenic mice exhibited high titres of anti-oligomer antibodies. However, this did not prevent but rather exacerbated beta cell apoptosis, leading to the conclusion that toxic oligomers may be intracellular and thus remain inaccessible to circulating antibodies [39].

A further indirect approach involved exploitation of the fact that various preparations of hIAPP have different cytotoxic potentials, which is thought to relate to the rate of oligomer formation. hIAPP preparations with low toxicity to RINm5F beta cells were found to contain predominantly mature fibrils in beta sheet conformation, whereas preparations with high toxicity contained fewer fibrils and were initially in random coil

state [50]. From such studies, it has been concluded that mature fibrillar hIAPP is not the toxic species. With the same principle in mind, the ability of hIAPP to induce apoptosis has been investigated in freshly prepared (primarily soluble hIAPP) vs pre-formed (mature) fibrillar preparations. When freshly dissolved peptide was added to dispersed islet cells from either humans or mice, a reduction in cell viability was observed, as well as necrosis and apoptosis, consistent with the idea that soluble hIAPP rapidly associates to form the putative toxic oligomeric species [51]. Interestingly, in this latter study the detrimental effect of fresh hIAPP was not seen in intact islets, and this was suggested to be due to an inability of the peptide to penetrate the outer islet membrane.

Taken together, the abovementioned studies are suggestive of an early hIAPP aggregate being involved in beta cell toxicity; however, more direct evidence is required and other possibilities have to be considered before the conclusion can be drawn that oligomers of hIAPP are the culprit.

Oligomers may not be the only cytotoxic species of islet amyloidosis

To date there has been only one report of anti-oligomer antibody immunoreactivity in association with beta cell apoptosis in human pancreas [40], although it is not entirely clear what the anti-oligomer antibody detected. In contrast, there are multiple reports of post-mortem studies demonstrating amyloid deposits in the majority of individuals with type 2 diabetes, with the proportion of affected individuals ranging from 40% to 90% [2–6, 52, 53] (lower estimates in some studies may be attributed to the use of Congo Red to detect amyloid, as this is less sensitive than thioflavin S [53]). Furthermore, the extent of amyloid deposition is positively associated with beta cell loss ([7] and R. L. Hull, unpublished observations). It is not inconceivable that large amyloid deposits in the extracellular space would cause significant structural damage to islets, disrupting blood flow and substrate passage, thereby inducing apoptosis. Indeed, it has been documented that degenerating islet cells lie in close proximity to aggregated hIAPP [13] and that inhibition of amyloid formation reduces beta cell apoptosis [54–56]. Certainly, cytotoxicity has been observed when pre-formed fibrils derived from hIAPP are applied to beta cells [32, 57], an effect that cannot be ascribed simply to oligomers.

Recently, sedimentation velocity studies of hIAPP have been performed at pH 4 to determine the predominant species in solution. This work showed that only monomers co-exist with mature fibrils and, perhaps surprisingly, oligomers containing fewer than 100 monomers could not be detected [58]. Similarly in another study utilising nuclear magnetic resonance, small oligomeric hIAPP (<100 nm in diameter) was not detected in solution at 4°C [59]. Together, these are significant observations; if the results can be translated to physiological conditions, they call into question the true identity of the putative oligomers. Reports documenting the size of oligomers formed by hIAPP are somewhat scarce; Green et al. determined hIAPP oligomers to comprise at least 16 monomers [43], while a size of 25–500 monomers was reported by Janson et al. [51] and 20–40 monomers by Anguiano et al. [60]. Further characterisation of hIAPP oligomers is needed, particularly to establish whether a specific size requirement exists for oligomer-mediated effects. In line with this, characterisation of the hIAPP oligomers that bind both the A11 and I11 antibodies would be extremely helpful. It has been shown that a minimum of eight monomers is required for detection of the A-beta(1–42) oligomer by the A11 anti-oligomer antibody [36]; however, the size of hIAPP oligomers detected by this anti-oligomer antibody has not been established.

Inhibitors of fibril formation have also been central in establishing whether soluble hIAPP oligomers are toxic. The main limitation, however, is that these studies have yielded conflicting data regarding exactly which form of hIAPP is inhibited. An example of such an inhibitor is rifampicin, an antibiotic that inhibits the aggregation and toxicity of A-beta(1–40) [61, 62]. Results from studies using rifampicin in the context of hIAPP have been conflicting. One report suggested that it inhibits fibril formation but not hIAPP oligomerisation or cytotoxicity [29], implying that oligomers are the toxic species and that they form via an off-pathway. Conversely, it has been reported that rifampicin does not inhibit amyloid formation [31, 32] but does inhibit toxicity of aggregated hIAPP [32]. Of note, recent work has clarified this

discrepancy by demonstrating that rifampicin interferes with fluorescence-based assays of amyloid formation, such as thioflavin T, probably by preventing thioflavin T from binding to hIAPP fibrils or quenching the fluorescence of bound thioflavin T [31]. Thus, fibril formation still occurs in the presence of rifampicin as previously reported [31, 32] and is detectable by electron microscopy but not by thioflavin T fluorescence. The clear implication of these findings is that conclusions drawn from studies using rifampicin in thioflavin T assays, including the suggestion of the existence of an off-pathway for hIAPP oligomer formation and cytotoxicity, have to be seriously re-evaluated, and will require validation by some other means.

How does hIAPP aggregation cause cytotoxicity?

The potential mechanisms for hIAPP-induced cytotoxicity have been explored and used to differentiate whether the actions of oligomers and/or mature fibrils are responsible for cell death. Perhaps the most widely accepted hypothesis is that hIAPP-induced cytotoxicity occurs via a membrane disruption mechanism: hIAPP oligomers can result in membrane leakage via direct interaction and/or formation of ion channels/pores (reviewed in [63]), while fibril growth has been implicated in membrane damage, possibly via interaction of fibrils with specific channels located on the cell surface [64]. By comparing the kinetics of both hIAPP fibril growth (using the thioflavin T assay) and membrane leakage, it was demonstrated that the kinetic profile of membrane leakage was characterised by an initial lag phase, followed by a sigmoidal transition, thereby matching the profile of fibril growth [65] (Fig. 1). When the initial nucleation process of fibril formation was accelerated by performing the membrane leakage assay in the presence of seeds of preformed hIAPP fibrils, the lag time of membrane damage was reduced. Of note, seeding is not thought to accelerate oligomer formation [66], and would not be expected to increase the presence of an off-pathway oligomeric form of hIAPP. Thus, these kinetic data argue against oligomers per se being responsible for membrane disruption, and rather point to fibril growth (i.e. elongation of hIAPP fibrils) as the cause. In line with this, another study utilising hIAPP transgenic mice demonstrated a positive correlation between the frequency of beta cell apoptosis and the change in islet amyloid area over time, but not the extent of amyloid deposition [47]. In this latter study it was also concluded that the process of amyloid formation, rather than the amyloid deposits specifically, may be cytotoxic.

The apoptotic signals triggered by hIAPP-induced insults such as membrane disruption also remain to be fully established. It has been documented that following exposure of immortalised beta cells or rodent islets to hIAPP, there is activation of multiple apoptotic pathways (caspase-8, JNK, p38 kinase and Fas-associated signalling pathways) [67–70]. The limitation to most of these studies is that hIAPP is applied extracellularly and thus the findings may not necessarily be consistent with apoptotic mechanisms because of endogenous production and subsequent aggregation of hIAPP. This said, the available data are compatible with the hypothesis that aggregating hIAPP is involved in beta cell toxicity—this may include oligomeric and/or fibrillar hIAPP.

Considerations and challenges for future work

The main evidence in favour of toxicity of hIAPP oligomers is summarised in the textbox (Current evidence for the toxicity of hIAPP oligomers per se or another species/event during islet amyloid formation in type 2 diabetes), along with other evidence that argues for the toxicity of hIAPP aggregation in general. Much of the latter evidence does not indicate a role of one specific species in beta cell toxicity, and so the idea that oligomers are involved is not excluded. The weight to be placed on each line of evidence requires careful consideration in line with the strengths and limitations of each study as discussed in detail in the text. Overall, it is apparent that no one piece of evidence provides a strong case for or against the toxicity of hIAPP oligomers in vivo. Thus, it is clear that the cause of beta cell death during islet amyloid formation remains debatable. If the process of amyloid formation in vivo is a continuum, with monomeric hIAPP aggregating to eventually form amyloid deposits, oligomers must also form in vivo. While mature amyloid fibrils may not be the primary toxic species, we submit that there is clearly insufficient evidence to accept oligomers as the sole and most important culprit.

Current evidence for the toxicity of hIAPP oligomers per se or another species/event during islet amyloid formation in type 2 diabetes

Evidence for the toxicity of hIAPP oligomers

- Beta cell toxicity occurs in the absence of fibrils
- Synthetic hIAPP in beta sheet conformation has low toxicity; hIAPP in random coil state has high toxicity
- hIAPP oligomers interact directly with membranes or form pores, resulting in leakage
- Multiple apoptotic pathways are activated by treatment with fresh soluble synthetic hIAPP

Evidence for the toxicity of hIAPP aggregation (may include oligomeric and/or fibrillar species)

- Pre-formed hIAPP fibrils induce beta cell apoptosis
- Inhibitors of amyloid formation reduce beta cell apoptosis
- Kinetic profile of membrane leakage matches that of hIAPP fibril growth
- Degenerating islets lie in close proximity to aggregated hIAPP
- Extent of amyloid deposition is associated with beta cell loss

While the field has advanced somewhat in its ability to detect and discriminate between various IAPP species, a number of deficiencies exist. Perhaps most critical is the limited number of simple reliable tools available and the lack of information regarding their specificity. Development of molecules that can slow the rapid aggregation of hIAPP may prove useful in identifying the species responsible for toxicity. Also, combining such molecules with the application of already available high-resolution microscopy techniques and cross-linker methods in studies utilising in vivo models of islet amyloid formation may provide valuable and specific information regarding the toxic species. Strikingly, a great deal of the literature supporting the hypothesis that oligomers derived from hIAPP are the main cytotoxic species has described work performed using the A11 antibody raised against A-beta oligomers. This antibody has not been extensively validated for use in different assays involving hIAPP oligomer formation. Further, it does not specifically detect pathogenic amyloid oligomers as it also binds other natively folded proteins found in islets.

There is also confusion about what constitutes an oligomer as the term has been used loosely to describe any species that is not fibrillar and/or cannot be detected by the usual means of detecting fibrillar deposits (thioflavin and electron microscopy). In its strictest sense, an oligomer is a non-covalent association of two or more monomers. As hIAPP oligomers have not been extensively characterised, particularly in humans, then the term ‘oligomer’ may not adequately reflect the structures which are involved in hIAPP fibril assembly and beta cell cytotoxicity. One could unify many examples in the literature where neither fibrils nor oligomers were truly and conclusively measured, yet findings suggest beta cell cytotoxicity had occurred, as a result of a form of hIAPP that is beyond monomeric yet precedes an organised amyloid deposit.

Another important consideration is the applicability of in vitro oligomer studies to hIAPP toxicity in vivo. Synthetic hIAPP is known to be aggressively amyloidogenic in vitro and experimental conditions can greatly influence aggregation [71], thus making studies of intermediates exceptionally difficult unless conditions are optimised to ensure hIAPP remains in the desired conformation. Moreover, different hIAPP preparations obtained from the same commercial source, but differing in lot numbers, can exhibit vastly different fibrillogenic and cytotoxic potential [50]. This variability in hIAPP preparations is an important factor that could potentially explain some of the discrepancies in data between in vitro studies. In addition, synthetic hIAPP forms fibrils readily in the absence of other compounds and one-time extracellular application acutely induces detrimental effects. In contrast, in healthy humans, endogenous IAPP both in plasma and in the beta cell remains in soluble form, with aggregation observed as amyloid deposits occurring over a protracted period in individuals with disturbed glucose homeostasis or insulinomas [2, 5, 72, 73]. Specific conditions (e.g. changes in secretory granule pH and calcium concentration) and cofactors (e.g. heparan sulphate proteoglycans) are associated with aggregation of endogenous IAPP. Thus separation of the toxic effects of oligomers vs fibrils and, in fact, demonstration of the involvement of oligomers per se in the face of many other cellular perturbations is substantially more difficult. Finally, in vivo models of islet amyloid formation have employed primarily hIAPP transgenic

rodents; however, many overproduce the peptide in quantities that far exceed those observed in humans and this itself may promote toxicity. Further, some of these models do not develop the typical deposits observed in humans [45, 46, 74].

In conclusion, the literature indicates that the mechanism responsible for beta cell cytotoxicity during the process of islet amyloid formation is still largely unknown. Knowledge gained from studies of A-beta oligomers in Alzheimer's disease may not necessarily apply to amyloidosis in type 2 diabetes given that a number of noteworthy differences in amyloidogenesis exist between the two diseases. This said, the idea that oligomers derived from hIAPP are primarily responsible is attractive; however, adopting it as dogma would seem rather premature. Development of more sophisticated yet practical approaches for the study of islet amyloidogenesis, particularly in vivo, are vital to advance the field and provide greater insight into the mechanism of hIAPP-mediated beta cell loss in type 2 diabetes.

Acknowledgements The authors are supported by the Medical Research Service of the Department of Veterans Affairs (S. Zraika, R. L. Hull, S. E. Kahn), the Canadian Diabetes Association (C. B. Verchere), the Canadian Institutes of Health Research (P. E. Fraser) and the Alzheimer Society of Ontario (P. E. Fraser). They are also in receipt of the following grants: NIH grants DK-075998 to S. E. Kahn, DK-074404 to R. L. Hull, DK-080945 to S. Zraika and GM-078114 to D. P. Raleigh; a Canadian Institutes of Health Research grant MOP-14682 to C. B. Verchere; a Juvenile Diabetes Research Foundation Postdoctoral Fellowship grant to S. Zraika; and a Canadian Institutes of Health Research R&D Studentship to K. J. Potter. C. B. Verchere is a Senior Scholar of the Michael Smith Foundation for Health Research.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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