Islet Amyloid Polypeptide (IAPP) Transgenic Rodents as Models for Type 2 Diabetes

Aleksey V. Matveyenko and Peter C. Butler

Abstract

Blood glucose concentrations are maintained by insulin secreted from β-cells located in the islets of Langerhans. There are ~2000 β-cells per islet, and ~one million islets of Langerhans scattered throughout the pancreas. The islet in type 2 diabetes mellitus (T2D) has deficient β-cell mass due to increased β-cell apoptosis and islet amyloid derived from islet amyloid polypeptide (IAPP). Accumulating evidence implicates toxic IAPP oligomers in the mediation of β-cell apoptosis in T2D. Humans, monkeys, and cats express an amyloidogenic toxic form of IAPP and spontaneously develop diabetes characterized by islet amyloid deposits. However, longitudinal studies of islet pathology in humans are impossible, and studies in nonhuman primates and cats are costly and impractical. Rodent IAPP is not amyloidogenic, thus commonly used rodent models of diabetes do not recapitulate islet pathology in humans. To investigate the diabetogenic role of human IAPP (h-IAPP), several mouse models and, more recently, a rat model transgenic for h-IAPP have been developed. Studies in these models have revealed that the toxic effect of h-IAPP on β-cell apoptosis demonstrates a threshold-dependent effect. Specifically, increasing h-IAPP transgene expression by breeding or induction of insulin resistance leads to increased β-cell apoptosis and diabetes. These transgenic rodent models for h-IAPP provide an opportunity to elucidate the mechanisms responsible for h-IAPP-induced β-cell apoptosis further and to test novel approaches to the prevention and treatment of T2D.

Key Words: amyloid; apoptosis; beta-cell; diabetes; h-IAPP transgenic mouse; HIP rat; islet amyloid polypeptide (IAPP)

Type 2 Diabetes

Worldwide more than 100 million people have been diagnosed with type 2 diabetes mellitus (T2D1), and it has been estimated that many millions still remain undiagnosed and untreated (King et al. 1998). This common disease compromises both quality and length of life, and places a substantial burden on health care systems. Macrovascular complications include increased risk of heart attack, stroke, and leg amputation (UKPDS 1998; Wei et al. 1998). Microvascular changes may lead to blindness, kidney failure, and limb-threatening neuropathy (UKPDS 1998). The direct costs of providing health care for people with T2D in the United States alone is estimated at ~$130 billion a year. Whereas T2D used to be a disease of midlife or old age, it is increasingly seen in children and young adults (Fagot-Campagna et al. 2001; Rosenbloom et al. 1999). Lifelong prevention of microvascular and macrovascular complications of diabetes in such young people is both expensive and unlikely to be successful.

Clearly a priority is to develop strategies to prevent development of T2D. To meet this objective, it is necessary to establish the mechanisms underlying the development of T2D.

Pathophysiology and Islet Pathology in Type 2 Diabetes

T2D has a strong but complex genetic component. Various risk factors are recognized, including obesity (Chan et al. 1994; Warne et al. 1995), hepatic cirrhosis (Simo et al. 1996), long-term use of corticosteroid treatment or Cush- ing’s disease (Boscaro et al. 2001), acromegaly (Hansen et al. 1986; Luft et al. 1967), spinal cord disruption, and muscular dystrophies (Frisbie 2005; Perseghin et al. 2003). All of these risk factors have insulin resistance in common. However, most insulin-resistant people (e.g., obese individuals) do not develop T2D (Chan et al. 1994), implying that insulin resistance induces T2D in those people who are genetically predisposed.

In humans with T2D glucose-induced insulin secretion is markedly diminished (Ward et al. 1984), although to some extent this reduction is masked by decreased hepatic insulin clearance (Sando et al. 1980). In health, the islets of Langerhans are a highly organized micro-organelle adapted to sense circulating nutrients (particularly glucose) and secrete insulin accordingly. Each islet (there are approximately one million in humans) is independently vascularized, and the afferent blood supply serves first the β-cell enriched core and then the predominantly α-cell mantle. The islet in T2D is characterized by ~65% decrease in the number of insulin-secreting β-cells, increased β-cell...
apoaptosis, and islet amyloid (Butler et al. 2003a). Islet amyloid in T2D is composed of extracellular fibrils of islet amyloid polypeptide (IAPP), a 37-amino acid protein that is coexpressed and cosecreted with insulin by β-cells (Butler et al. 1990). The sequence of IAPP displays close homology in its amino and carboxy terminal residues, whereas residues 20 through 29 show some variance between species (Figure 1). IAPP20-29 confers IAPP its amyloidogenic properties. Human, nonhuman primate, and feline IAPP is amyloidogenic, but rodent IAPP is not (Betsholtz et al. 1989; Westermark et al. 1990). It is therefore interesting that humans, monkeys, and cats spontaneously develop T2D characterized by islet amyloid while rodents do not (O’Brien et al. 1993).

These observations suggest that the increased β-cell apoptosis in T2D may in some way be related to the formation of islet amyloid, although evidence to date is necessarily indirect. In support, human IAPP (but not rodent IAPP) induces apoptosis when added to cells in culture (Janson et al. 1999; Lorenzo et al. 1994; Ritzel and Butler 2003). Once hyperglycemia supervenes, hyperglycemia per se is known to induce β-cell apoptosis (Maedler et al. 2002). In addition, high free fatty acid concentrations have also been implicated in increased β-cell apoptosis in T2D (Cnop et al. 2001; Shimabukuro et al. 1998). Taken together, these data suggest that in those individuals genetically predisposed, in response to insulin resistance there is a progressive loss of β-cells initially possibly due to mishandling of IAPP and subsequently also due to hyperglycemia and increased FFA characteristic of T2D. This model emphasizes the need for further study of the initiation and early loss of β-cells in T2D.

### Commonly Used Rodent Models of Type 2 Diabetes

Longitudinal studies of pancreas morphology are impossible in humans. Usually human pancreas becomes available only at autopsy or during surgical resection for pancreatic cancer. The former is complicated by autolysis (Shimizu et al. 1990) and the latter by changes in islet function and anatomy resulting from pancreatic cancer (Fogar et al. 1994). Animals that spontaneously develop T2D include nonhuman primates and cats (Howard 1986; O’Brien et al. 1993); however, only a small and unpredictable proportion of these animals develop T2D, so diabetes prevention studies would require costly maintenance of huge colonies of primates over many years.

Rodent models circumvent problems with pancreas availability and cost because it is possible to conduct longitudinal studies using larger numbers of animals. Nevertheless, putative rodent models of diabetes either do not fully recapitulate islet pathology in humans with T2D (Donath et al. 1999; Portha et al. 2001) or require an extreme obesity phenotype to develop diabetes (Lee et al. 1994; Shafrir et al. 1999; Tomita et al. 1992). Commonly used rodent models for T2D are the db/db mice and the diabetes-prone Zucker diabetic fatty rat (ZDF). Both models harbor mutations on leptin receptors (Chen et al. 1996; Phillips et al. 1996) and are overtly obese with a sudden onset of hyperglycemia within the first few months of life (Kawasaki et al. 2005; Lee et al. 1994). A similar murine model, the ob/ob mouse, has a mutation in the leptin gene (Pelley et al. 1996) and are overtly obese with a sudden onset of profound obesity and do not share the same islet pathology as humans with T2D (islet amyloid).

The high calorie-fed *Psammomys obesus* (sand rat) is another rodent model of T2D induced by onset of insulin resistance (Shafrir and Gutman 1993). This animal usually lives on a low-calorie diet of sage brush but develops hyperglycemia (>20 mmol/L) within a few days of being placed on a high energy diet, which induces marked insulin resistance (Shafrir et al. 1999; Tomita et al. 1992). The mechanism subserving failure of β-cell mass expansion is not fully understood, but it has been suggested to be the combination of the gluco- and lipo-toxicity and subsequent increase in β-cell apoptosis due to intra-islet triglyceride accumulation (Harmon et al. 2001; Lee et al. 1994; Unger 1995). All three models only develop diabetes in relation to profound obesity and do not share the same islet pathology as humans with T2D (islet amyloid).

### Islet Amyloid Polypeptide (IAPP)

<table>
<thead>
<tr>
<th>Human</th>
<th>KCONATCATQRLANFLVSNNFGAILSTNVSSNTFY</th>
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<tbody>
<tr>
<td>Monkey</td>
<td>R. T. D.</td>
</tr>
<tr>
<td>Cat</td>
<td>IR. L. P.</td>
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<tr>
<td>Mouse</td>
<td>R. L. PV. PP.</td>
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<tr>
<td>Rat</td>
<td>R. L. PV. PP.</td>
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*Figure 1* Amino acid sequence of islet amyloid polypeptide (IAPP) in different species.
2001). Characteristically, fasting blood glucose levels are only slightly elevated due to impaired insulin secretion and the presence of liver and skeletal muscle insulin resistance (Picarel-Blanchot et al. 1996). Although it is possible that there are forms of diabetes in humans due to inadequate β-cell proliferation in early life, there is no evidence for this to date.

Studies with the models of T2D listed in Table 1 have made invaluable contributions to the field of T2D. However, because all of the models have nonamyloidogenic rodent IAPP, it is not possible to evaluate the role of IAPP oligomerization in islet pathophysiology using these models. To circumvent this problem, several mouse models transgenic for human IAPP (h-IAPP) (Table 2) have been developed.

### Mouse Models Transgenic for h-IAPP

Because mouse IAPP is nonamyloidogenic due to proline residues in the amyloidogenic region of human IAPP, use of transgenic technology to create transgenic mouse models for human IAPP was an attractive approach to investigating the possible adverse effects of amyloidogenic IAPP on β-cell destruction. The rat insulin II promoter has been used to target human IAPP to mouse β-cells. The first such mouse model reported did not develop diabetes or islet amyloid but did have abnormal IAPP aggregates in β-cell secretory granules (De Koning et al. 1994). Subsequent mouse models with a comparable burden of transgenic human IAPP expression (heterozygotes) also did not develop diabetes (Couce et al. 1996; Wang et al. 2001; Yagui et al.

### Table 1 Commonly used rodent models of type 2 diabetes and their respective phenotype and islet pathology

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Metabolic Phenotype</th>
<th>Islet Pathology</th>
<th>References (See Text)</th>
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<tbody>
<tr>
<td><strong>db/db mouse</strong></td>
<td>Mice are hyperphagic and display hyperglycemia (∼20 mM glucose) and hyperlipidemia within the first month of life. Severe insulin resistance and impaired insulin secretion are also present.</td>
<td>1. Enlarged islets but decreased β-cell mass due to β-cell apoptosis/necrosis 2. Evidence for loss of β-cell differentiation capacity 3. Exhibit extensive intraislet lipid accumulation</td>
<td>Baetens et al. 1978; Kawasaki et al. 2005; Medarova et al. 2005; Shafrir et al. 1999</td>
</tr>
<tr>
<td><strong>ob/ob mouse</strong></td>
<td>Animals are hyperphagic, hyperglycemic, and hyperlipidemic. Mice are also markedly hyperinsulinemic to compensate for severe insulin resistance.</td>
<td>1. Marked islet hyperplasia in response to hyperglycemia with no change in islet number 2. Increase in islet insulin content presumably due to increase in β-cell number</td>
<td>Bock et al. 2003; Edvell and Lindstrom 1999</td>
</tr>
<tr>
<td><strong>Zucker diabetic fatty (ZDF) rat</strong></td>
<td>Animals are obese, hyperphagic, and insulin resistant and develop hyperglycemia and diabetes by 10 wk of age. Impaired insulin secretion.</td>
<td>1. Decreased β-cell mass due to increase β-cell apoptosis compared with nondiabetic obese Zucker rats 2. Increased β-cell proliferation 3. 10-fold increase in β-cell triglyceride content</td>
<td>Finegood et al. 2001; Harmon et al. 2001; Lee et al. 1994; Pick et al. 1998; Unger 1995</td>
</tr>
<tr>
<td><strong>Psammomys obesus (sand rat)</strong></td>
<td>Normally nondiabetic but develop diabetes (∼20 mM glucose) within first few days of being put on high-energy diet. Diabetes development is preceded by severe insulin resistance and hyperinsulinemia.</td>
<td>1. Decreased β-cell mass 2. Increased β-cell apoptosis 3. Decreased β-cell proliferation</td>
<td>Donath et al. 1999; Shafrir and Gutman 1993; Shafrir et al. 1999</td>
</tr>
<tr>
<td><strong>Goto-Kakisaki (GK) rat</strong></td>
<td>Animals are mildly hyperglycemic from birth (8-10 mM glucose). As adults exhibit insulin resistance and impaired glucose-stimulated insulin secretion</td>
<td>1. Decreased β-cell mass due to diminished beta-cell proliferation and neogenesis 2. No change in β-cell apoptosis</td>
<td>Movassat et al. 1997; Picarel-Blanchot et al. 1996; Portha et al. 2001</td>
</tr>
<tr>
<td>Species</td>
<td>Transgene/Background</td>
<td>Metabolic Phenotype</td>
<td>Islet Pathology</td>
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</table>
| Mouse   | h-IAPP(hemizygous)/FVB/N | Mice do not develop diabetes unless treated with growth hormone and dexamethasone. | 1. Small intra- and extracellular amyloid deposits  
2. Evidence of β-cell degeneration | Couce et al. 1996 |
| Mouse   | h-IAPP(homozygous)/FVB/N | Mice spontaneously develop hyperglycemia (~11mM glucose) and diabetes by 10-14 wk of age. | 1. Small intra- and extracellular amyloid deposits  
2. Evidence of decreased β-cell mass | Janson et al. 1996 |
| Mouse   | h-IAPP(hemizygous)/A^v/A | Progressive development of diabetes by ~ 15 wk of age in obese but not in lean transgenics | 1. Large extracellular amyloid deposits  
2. Decreased β-cell mass (~80%)  
3. Increased β-cell apoptosis (~10 fold)  
4. No change in β-cell neogenesis or replication | Butler et al. 2003b; Soeller et al. 1998 |
| Mouse   | h-IAPP(hemizygous)/C57BL/6J | Mice do not spontaneously develop diabetes. Mice exhibit impaired insulin secretion in response to oral glucose load. | 1. No reported presence of extracellular amyloid deposits  
2. Electron microscopy reveals aggregation of amyloid fibrils in β-cell secretory granules  
3. No change in β-cell mass | Ahren et al. 1998; de Koning et al. 1994; Hoppenber et al. 1999 |
| Mouse   | h-IAPP(hemizygous)/C57BL/6J | Mice do not spontaneously develop diabetes and display normal glucose tolerance in vivo. Mice show evidence of impaired glucose-induced insulin secretion in isolated islets. | 1. No extracellular amyloid deposits  
2. Reported formation of amyloid fibrils in β-cell secretory granules  
3. No change in β-cell mass | Tokuyama et al. 1997; Yagui et al. 1995 |
| Mouse   | h-IAPP(hemizygous)/C57BL/6 x DBA | Mice do not spontaneously develop diabetes, however, tend to demonstrate impaired glucose tolerance in response to IPGTT due to diminished insulin secretion. | 1. Presence of extracellular amyloid deposits  
2. Evidence for decrease in β-cell mass (~15%) | Hull et al. 2003; Hull et al. 2005; Wang et al. 2001 |
| Mouse   | h-IAPP(hemizygous)/ob/+ | Mice spontaneously develop diabetes and stay hyperglycemic (~15-20 mM glucose) during their life span compared with nontransgenic ob/ob mice. | 1. Presence of extensive extracellular amyloid deposits.  
2. Substantial decrease in β-cell mass | Hoppenber et al. 1999 |
| Rat     | h-IAPP(homozygous)/CD | Sudden onset of diabetes with the first 2 mo of age | 1. No extracellular amyloid deposition.  
2. Rapid decline in β-cell mass | Butler et al. 2004 |
| Rat     | h-IAPP(hemizygous)/CD | Gradual onset of diabetes by midlife ~5-10 mo of age | 1. Presence of extensive extracellular amyloid deposits.  
2. Decreased in β-cell mass (~50-80%)  
3. Increased β-cell apoptosis (~10-fold)  
4. Increased β-cell replication, with no change in neogenesis | Butler et al. 2004 |
was initially reported to induce diabetes (Hull et al. 2003), but in that report it is noted that control animals also had glucose levels in the diabetic range, suggesting that there were technical issues in blood sampling from mice. In a subsequent report with the same model, the blood glucose was no higher in the fat-fed h-IAPP transgenic mouse models than the controls (Hull et al. 2005), although the former did develop islet amyloid, again emphasizing that it is not the amyloid per se that induces β-cell death.

Another strategy that has been used to increase transgenic expression of human IAPP is to cross-breed hemizygous h-IAPP transgenic mice onto a mouse with an obese background. This strategy has been successfully accomplished using both the Avy/Agouti and ob/ob mouse models of obesity (Butler et al. 2003b; Hoppener et al. 1999; Soeller et al. 1998). The resulting mice develop diabetes and islet amyloid. The former has been studied prospectively over 50 wk and was the first model in which it was confirmed that the mechanism of decreased β-cell mass in these mice is increased β-cell apoptosis (Figure 3). Again it was shown by these prospective studies that β-cell apoptosis is not directly related to the extent of islet amyloid but that, interestingly, it is related to the rate of amyloid formation, implying that toxicity is mediated by a common precursor. These prospective studies also allowed insight into the increased vulnerability of replicating β-cells to β-cell apoptosis, so that the net impact on loss of β-cell mass is amplified.

IAPP in its soluble form has been shown to have an inhibitory effect on insulin secretion (Ohsawa et al. 1989; Tedstone et al. 1990). Thus, increased expression of IAPP in transgenic mice (the sum of endogenous IAPP and transgenic IAPP) may lead to hyperglycemia by inhibition of insulin secretion. Several of the human IAPP transgenic mouse models have shown impaired glucose-mediated insulin secretion that precedes loss of β-cell mass (Ahren et al. 1998; Hull et al. 2003; Tokuyama et al. 1997). Although it was also previously reported that IAPP may also induce insulin resistance (Leighton and Cooper 1990), this effect is not observed at the concentrations present in the circulation of these mouse models (nmol/L vs. pmol/L).

In summary, a variety of human IAPP transgenic mouse models have been developed. These studies have demonstrated that there is a dose effect of increasing human IAPP transgene expression on inducing β-cell toxicity. This toxicity appears to be related to small IAPP oligomers that are distinct from islet amyloid.

h-IAPP Transgenic Rat (HIP Rat)

The HIP rat is an h-IAPP transgenic model on the Sprague-Dawley background (Butler et al. 2004). Homozygous HIP rats developed diabetes rapidly within the first 2 mo of life, and hemizygous HIP rats spontaneously developed midlife diabetes (6-12 mo) associated with islet amyloid (Butler et al. 2004). These rats also provide further evidence for the concept of a threshold for expression beyond which h-IAPP
Figure 3 Representative islets from a lean nontransgenic (LNT) mouse (left column); obese nontransgenic (ONT) mouse (central column) and obese h-IAPP transgenic mouse (right column). The top panels are stained for insulin, the middle panels are stained for replication using Ki67 marker, and the bottom panels are stained for apoptosis using the TUNEL method. Increased islet size is evident in islets from OT and ONT mice, with extensive amyloid deposits in the islet from the OT mouse. Numerous replicating cells are present in islets from obese mice (OT and ONT), although none are present in islets from lean mice (LNT). Numerous apoptotic cells are present in OT mouse, as opposed to the islets from nontransgenic mice (LNT, ONT). From Butler AE, Janson J, Soeller WC, Butler PC. 2003b. Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: Evidence for role of islet amyloid formation rather than direct action of amyloid. Diabetes 52:2304-2314. Copyright © 1994 American Diabetes Association. From Diabetes, Vol. 52, 2003;2304-2314. Reprinted with permission from The American Diabetes Association.

Figure 4 Representative islets stained for insulin from a nondiabetic human and a wild-type rat (upper panel) and from a type 2 diabetic human and a 10-mo-old diabetic human islet amyloid polypeptide transgenic (HIP) rat (lower panel).
expression cannot be appropriately handled and trafficked, leading to oligomerization and toxicity. Multiple lines of h-IAPP transgenic rats were developed, and the propensity of these lines to develop diabetes was related to the expression of h-IAPP (Butler et al. 2004).

In prospective studies of the HIP rat, this model develops islet pathology closely related to that in humans (progressive loss of β-cell mass, islet amyloid, and increased β-cell apoptosis) (Figures 4 and 5) (Butler et al. 2004). Increased β-cell apoptosis and the deficit in β-cell mass precede development of hyperglycemia. The ~60% deficit in β-cell mass at the onset of diabetes in the HIP rat is comparable to the 65% β-cell mass loss observed in humans with T2D (Butler et al. 2003a). Once hyperglycemia develops in the HIP rat, β-cell apoptosis increases further and is correlated with the blood glucose concentration, implying glucose toxicity. The HIP rat model will provide an opportunity to evaluate the progression of abnormalities in insulin secretion and action in relation to changes in β-cell mass.

**Summary**

The islet in type 2 diabetes is characterized by islet amyloid derived from IAPP and ~60% deficit in β-cells due to increased β-cell apoptosis (Butler et al. 2003a). There is increasing interest in the potential role of the mishandling of IAPP in the pathophysiology of type 2 diabetes and whether it is possible to inhibit any such effect. Because rodent IAPP does not have the propensity to form IAPP oligomers or amyloid (Betsholtz et al. 1989), most rodent models are not useful in this respect. Although cats and monkeys do spontaneously develop type 2 diabetes characterized by loss of β-cell mass and islet amyloid (Howard 1986; O’Brien et al. 1993), the disease is sporadic and unpredictable so that prospective studies on diabetes prevention would require keeping large numbers of animals for many years. To overcome these difficulties, both murine and more recently rat transgenic models for human IAPP have been developed. Lessons that can be inferred from these in vivo data include the presence of a dose effect for human IAPP expression on β-cell toxicity, such that human IAPP is trafficked and secreted with no adverse effects below a threshold expression rate but thereafter there is increased β-cell apoptosis. Second, IAPP toxicity appears to be associated with the formation of small intracellular IAPP oligomers rather than the large extracellular islet amyloid deposits that may develop subsequently. Finally, human IAPP-induced β-cell apoptosis appears to also act to prevent recovery of β-cell mass since replicating β-cells have increased vulnerability to IAPP induced apoptosis. The specific mechanisms by which IAPP oligomers induce β-cell apoptosis and how this might be prevented remain to be established. Transgenic rodent models should be helpful in resolving these questions.

**Acknowledgments**

We gratefully acknowledge funding for these studies from the United States Public Health Service (NIH DK59579) and the Larry L. Hillblom Foundation. We also gratefully acknowledge the helpful suggestions from the following collaborators: At the Larry L. Hillblom Islet Research Center, University of California, Los Angeles, Anil Bhushan, Alexandra Butler, Tatyana Gurlo, Leena Haataja, and Kathrin Maedler; and at Pfizer Research in Groton CT, Walter Soeller.

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