Replication Increases β-Cell Vulnerability to Human Islet Amyloid Polypeptide–Induced Apoptosis

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Type 2 diabetes is characterized by a relative β-cell deficit as a result of increased β-cell apoptosis and islet amyloid derived from the β-cell peptide islet amyloid polypeptide (IAPP). Human IAPP (h-IAPP) but not mouse IAPP (*m*-IAPP) induces apoptosis when applied to cells in culture, a property that depends on the propensity of h-IAPP to oligomerize. Since β-cell mass is regulated, the question arises as to why it is not adaptively increased in response to insulin resistance and hyperglycemia in type 2 diabetes. This adaptation might fail if dividing β-cells preferentially underwent apoptosis. We tested the hypothesis that β-cells are preferentially vulnerable to h-IAPP–induced apoptosis. We established a microculture environment to perform time-lapse video microscopy (TLVM) and studied β-cells (RIN) and HeLa cells undergoing replication or apoptosis. Sequential images (every 10 min for 36 h in RIN or 24 h in HeLa cells) of cells in vivo were analyzed, and each mitotic and apoptotic event was documented. Freshly dissolved h-IAPP caused a dose-dependent increased rate of apoptosis ($P < 0.0001$) in both cell types. At low and medium levels of toxicity, cells that had previously undergone mitosis were more vulnerable to h-IAPP–induced apoptosis than nondividing cells ($P < 0.05$). In the first 3 h after mitosis (full cell cycle length 26 ± 0.6 h), β-cells were particularly susceptible to h-IAPP–induced apoptosis ($P < 0.05$). Neither *m*-IAPP nor mature amyloid aggregates of h-IAPP were cytotoxic ($P = 0.49$). To corroborate these cell culture studies, we examined sections of human pancreatic tissue (five cases of type 2 diabetes) and human islets incubated for 48 h ± h-IAPP. Both were stained for apoptosis with the transferase-mediated dUTP nick-end labeling method and analyzed for the presence of paired apoptotic cells anticipated in the event of postmitotic apoptosis. In human pancreatic tissue 26 ± 5% (single plane of examination) and in human islets incubated with h-IAPP 44 ± 4% of apoptotic islet cells were paired. In conclusion, replicating β-cells are preferentially vulnerable to h-IAPP–induced apoptosis in cell culture. Postmitotic apoptosis was also documented in humans with type 2 diabetes and in human islet tissue. We postulate that β-cell deficiency in type 2 diabetes may result in part from failure to adaptively increase β-cell mass due to increased vulnerability of replicating β-cells to undergo apoptosis. If this postulate is correct, then inhibition of apoptosis should allow recovery of β-cell mass in type 2 diabetes. *Diabetes* 52:1701–1708, 2003

Type 2 diabetes is characterized by partial β-cell loss (1–3) and islet amyloid derived from the 37–amino acid peptide islet amyloid polypeptide (IAPP) (4–8). IAPP is coexpressed with insulin by β-cells and trafficked with insulin to secretory vesicles (9) from which it is co-secreted (10–12). IAPP expression increases with insulin resistance (13,14). Human IAPP (h-IAPP) but not mouse IAPP (*m*-IAPP) is amyloidogenic in an aqueous environment (15). Humans but not mice may develop islet amyloid associated with diabetes when exposed to chronic insulin resistance. However, mice that express high levels of h-IAPP by being bred either to have a high IAPP transgene copy number or to be insulin-resistant through high-fat feeding (16), treatment with growth hormone and glucocorticoids (13), or genetic obesity develop diabetes characterized by islet amyloid (17–19). Taken together, these data suggest that there may be a link between the amyloidogenic properties of h-IAPP and loss of β-cell mass in type 2 diabetes.

This potential link was further highlighted by studies revealing that h-IAPP but not *m*-IAPP induces apoptosis when added to β-cells (20–22). Similarly, overexpression of h-IAPP but not *m*-IAPP in cell models leads to IAPP aggregation and cell death through apoptosis (23). Furthermore, recent studies suggest that the mechanism by which h-IAPP leads to cell toxicity is by formation of nonselective ion channels in cell membranes (24) by the early smaller or intermediate-sized particles of aggregating IAPP (intermediate-sized toxic particles) (25,26).

It has become increasingly apparent that β-cells replicate and that β-cell mass is regulated (27,28). For example, β-cell mass in rodents increases in response to insulin resistance and hyperglycemia (29,30). In obese nondiabetic humans β-cell mass increases, presumably in response to insulin resistance induced by obesity (1,3,31). It is therefore unclear why the β-cell mass in patients with type 2 diabetes does not increase in response to the chronic insulin resistance and hyperglycemia present in this disease (32). One explanation might be that cell replication increases the vulnerability to cytotoxic stimuli (21,33), and β-cell mass in subjects with type 2 diabetes does not adaptively increase to meet demands. In the present study, we therefore postulated that mitotic cells are more vulnerable to IAPP-induced apoptosis than non-

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IAPP, islet amyloid polypeptide; h-IAPP, human IAAP; *m*-IAPP, mouse IAPP; TLVM, time-lapse video microscopy; TUNEL, transferase-mediated dUTP nick-end labeling.

mitotic cells. To test this hypothesis, we developed a time-lapse video microscopy (TLVM) system that enables direct imaging of living cells in culture so that all mitotic and cell death events can be documented in a time-dependent manner.

RESEARCH DESIGN AND METHODS

Design. To address the hypothesis posed, we first established a microculture environment in which TLVM could be performed. To ensure that this system would allow maintenance of viable cells over the period of observation (24–36 h), we first ensured that pH and temperature of the culture medium were stable for this period. Second, we established two cell lines (RIN and HeLa) that replicated with sufficient frequency and remained adherent to the cell culture dish in a uniform monolayer, therefore allowing us to observe frequent mitotic events. Third, we used these methods to establish the rate of cell death and replication in the presence and absence of freshly prepared versus mature h-IAPP solutions. Previous studies have shown that the toxic particles of h-IAPP are present in recently dissolved h-IAPP (dissolved within 2 h of application) but not in matured solutions of h-IAPP (dissolved at least 5 days before study) (25). In the present studies, we examined the effects of various concentrations of freshly dissolved h-IAPP (0, 20, 40, and 80 μmol/l) on cell viability. Also, as controls, we examined the effects of mature solutions (40 μmol/l) and *m*-IAPP (40 μmol/l) on cell replication and death rates.

Cell lines. HeLa cells (strain CCL-2; ATCC, Manassas, VA), a human cell line derived from cervical cancer, were maintained in culture in Click's culture medium with 10 mmol/l glucose and 10% FBS at 37°C in humidified air containing 5% CO_2. After trypsin digestion of the primary cell culture, aliquots of suspended cells were placed in a specially prepared microculture dish (2.3-cm diameter, ΔT Culture Dish; Bioptechs, Butler, PA) in which they formed a partial monolayer (~30%) over the following 24 h cultured in a conventional incubator (Model 3110; Forma Scientific, Marjetta, OH). These cells could be repetitively imaged by TLVM (as they remained in a single cell layer and did not develop multilayer clusters as occurs with β-cell lines); and frequent cell divisions were observed.

RIN cells (a gift from C.J. Rhodes, Pacific Northwest Research Institute, Seattle, WA), a rodent β-cell line, were maintained under identical conditions as described above for HeLa cells. To ensure that the β-cells would not form multilayer clusters, we coated the microculture dishes for TLVM with a human tumor bladder-9 matrix, and the culture medium was supplemented with 20 ng/ml HGF/SF to increase the scatter effect (34). HeLa cells were cultured without the human tumor bladder-9 matrix and HGF/SF because they have the inherent characteristic to grow in a monolayer in glass culture dishes (as used for the incubation chamber on the microscope).

Human pancreatic tissue. Human pancreatic tissue was obtained at autopsy from five subjects with type 2 diabetes. Adjacent tissue sections were stained for insulin (immunostaining) and apoptosis (transferase-mediated dUTP nick-end labeling [TUNEL] technique). TUNEL-positive cells that corresponded to insulin-positive tissue in the adjacent section were counted per islet. To address the question of whether replicating β-cells are particularly vulnerable to apoptosis, we assessed the fraction of apoptotic cells organized in pairs. TUNEL-positive cells that were within less than a single cell width from each other on cross-section were counted as a pair. A total of 6 to 10 islets were analyzed per subject. The β-cell area and apoptosis frequency of these human pancreatic sections are part of a previously published study (3).

Human islet tissue. Human pancreatic islets were isolated from the whole pancreas retrieved from one heart-beating organ-donor by the Diabetes Institute for Immunology and Transplantation, University of Minnesota (Bernhard J. Hering). The donor did not have a history of diabetes. After isolation, islets were maintained in RPMI culture medium (5 mmol/l glucose) supplemented with 10% FBS at 37°C in humidified air containing 5% CO_2. Experiments were performed after a recovery period of 3 days following the islet isolation process. Aliquots of human islets were incubated for 48 h with vehicle, 40 μmol/l rat IAPP, or 40 μmol/l human IAPP. The number of apoptotic cells in human islets was detected using the TUNEL method (In Situ Cell Death Detection Kit, AP; Roche Diagnostics, Indianapolis, IN). Identical to the analysis of human pancreatic autopsy tissue, TUNEL-positive cells that were within less than a single cell width from each other on cross-sectional area were counted as a pair.

TLVM. For TLVM experiments, the microculture dish was removed from the regular incubator and mounted onto the motorized stage (H107, ProScan; Prior Scientific) of an inverted microscope (Inverted System Microscope IX 70; Olympus, Melville, NY). The microculture dish was specially designed with an optically transparent, electrically conductive indium tin-oxide coating on the bottom surface to provide direct heat transfer to cells. The temperature inside the dish was dynamically controlled to 37 ± 0.1°C (ΔT Culture Dish Controller; Bioptechs). The dish was covered with an electrically heated, optically transparent lid to prevent condensation from forming on the under surface of the cover. pH was stabilized by the inflow of air containing 5% CO_2. The pH of the culture medium was recorded in separate experiments with a pH microelectrode (PHR-146; Lazar Research Laboratories, Los Angeles, CA) and remained stable throughout the 24- to 36-h observation period. For time-lapse experiments, images of selected fields were acquired with an analogue camera (3-CCD camera; Optronics) every 10 min, stored, and analyzed on a personal computer (Intel Pentium processor, 700 MHz; Image-Pro Plus and Scope Pro software).

IAPP. Human and mouse IAPPs were purchased from Bachem California (Torrance, CA). For the preparation of mature human IAPP in amyloid form, lyophilized IAPP was dissolved in deionized water (0.5% acetic acid) to prepare a stock solution. The stock solution was then diluted with the culture medium to obtain the desired final IAPP concentrations. This preparation was stored for 5 days at 4°C to allow fibril formation to occur.

When applying fresh IAPP, the stock solution was diluted with the culture medium, which was then applied directly to the cells within 3 min. Acetic acid concentrations in the culture medium applied to cells were always <0.003%.

Assessment of IAPP effects on cell death and cell division. The rate of cell division, cell death, and the net cell number were assessed by examining the merged image sequences obtained over 24 h in HeLa cells (n = 2,591) and RIN cells (n = 1,148). Images were acquired from at least four separate fields (locations in the culture chamber) during each experiment. Mitosis and apoptosis both showed characteristic morphological changes when observed with TLVM in both cell types. A successful cell division was judged to have occurred when a single cell divided into two daughter cells (Fig. 1*A*). Apoptosis was judged to have occurred when a cell rounded up, the nucleus condensed and subsequently fragmented, and the cell cytoplasm disintegrated via cytoplasmic blebs into apoptotic bodies (Fig. 1*B*).

To establish the impact of cell division on the vulnerability of cells to IAPP-induced apoptosis, we assigned the cells into groups of *1*) dividing cells, if a cell division event occurred, versus *2*) nondividing cells. Each individual cell was tracked until one of three events occurred: cell replication, cell death, or the end of the experiment to calculate the relative fraction of dividing and nondividing cells undergoing apoptosis. The time intervals between replication and subsequent apoptosis in the same cell (Fig. 1*C*) were additionally analyzed by plotting their frequency distribution in comparison with the full cell cycle length to establish when during the cell cycle the cells were most vulnerable to IAPP-induced apoptosis. To continue TLVM imaging over a full cell cycle length in the majority of cells, only replicating cells from the first 6 h (HeLa) or 12 h (RIN) of the experiments were included for this analysis. Therefore, RIN cells were imaged over a total period of 36 h and HeLa cells for 24 h.

Statistical analysis. Changes in the mean rates of cell death, cell division, and net cell number after each treatment were determined by the Student's t test. Changes in the rates of these processes with time were examined by use of ANOVA. The histograms of the time interval between apoptosis and cell replication in the same cell were analyzed by linear regression. A probability of <5% occurrence by chance alone denoted statistical significance.

RESULTS

Stability of cell culture conditions with TLVM technique. Temperature was stable at 37.0 ± 0.3°C. pH was documented in separate experiments and was stable over the 24- to 36-h experimental period. Cells cultured under control conditions without IAPP were viable over the whole experimental period and underwent cell replication (Fig. 1*A*). There was a linear growth of the cell population (Figs. 2*A* and 3*A*) resulting from a stable rate of mitosis (Figs. 2*B* and 3*B*), which exceeded the rate of apoptosis (Figs. 2*C* and 3*C*). The mean cell cycle length was 26 ± 0.6 h in RIN cells and 20 ± 0.3 h in HeLa cells.

Effects of IAPP on cultured RIN cells. Freshly dissolved h-IAPP was dose-dependently cytotoxic on cultured β-cells (Fig. 2, right). Cytotoxicity was first evident 14–18 h after application of h-IAPP. Nonamyloidogenic *m*-IAPP or aggregated h-IAPP did not induce increased rates of cell death (Fig. 2, left). Freshly dissolved h-IAPP, matured h-IAPP, and *m*-IAPP had no significant effect on the rate of β-cell replication.

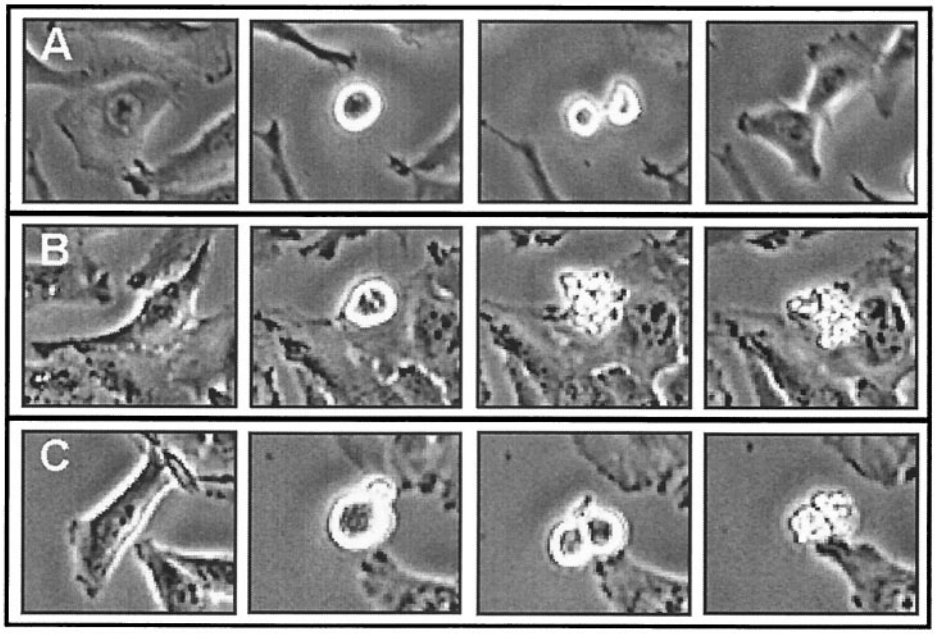

FIG. 1. ***A*****: Cell replication: a flattened HeLa cell is rounding up and dividing into two daughter cells, which reestablish to the culture dish.** ***B*****: Apoptosis in nondividing cells: a flattened cell is rounding up and is at this point morphologically identical to the cell in** ***A*****; the cell in** ***B*** **in contrast undergoes apoptosis with classical morphological changes such as blebbing of the plasma membrane, shrinkage, and fragmentation into apoptotic bodies.** ***C*****: Apoptosis in replicating cells: a flattened cell is rounding up and dividing into two daughter cells that then undergo apoptosis.**

Effects of IAPP on cultured HeLa cells. Application of freshly dissolved h-IAPP led to a dose-dependent increased rate of apoptosis (Figs. 1*B* and *C* and 3, right) in HeLa cells. These cell death events were first evident ~4–6 h after application (Fig. 3). Neither *m*-IAPP nor matured solutions of h-IAPP caused increased apoptosis compared with control (Fig. 3, left). Freshly dissolved h-IAPP dose-dependently reduced the rate of HeLa cell replication.

Increased vulnerability of replicating cells in cell lines to h-IAPP. Analysis of the merged images from TLVM revealed that cells previously undergoing mitosis were most vulnerable to h-IAPP apoptosis. We examined the relationship between apoptotic events and the presence and timing of previous mitotic events as described in RESEARCH DESIGN AND METHODS at each concentration of h-IAPP applied. The results indicate that at low and medium levels of cytotoxicity, HeLa cells (20 and 40

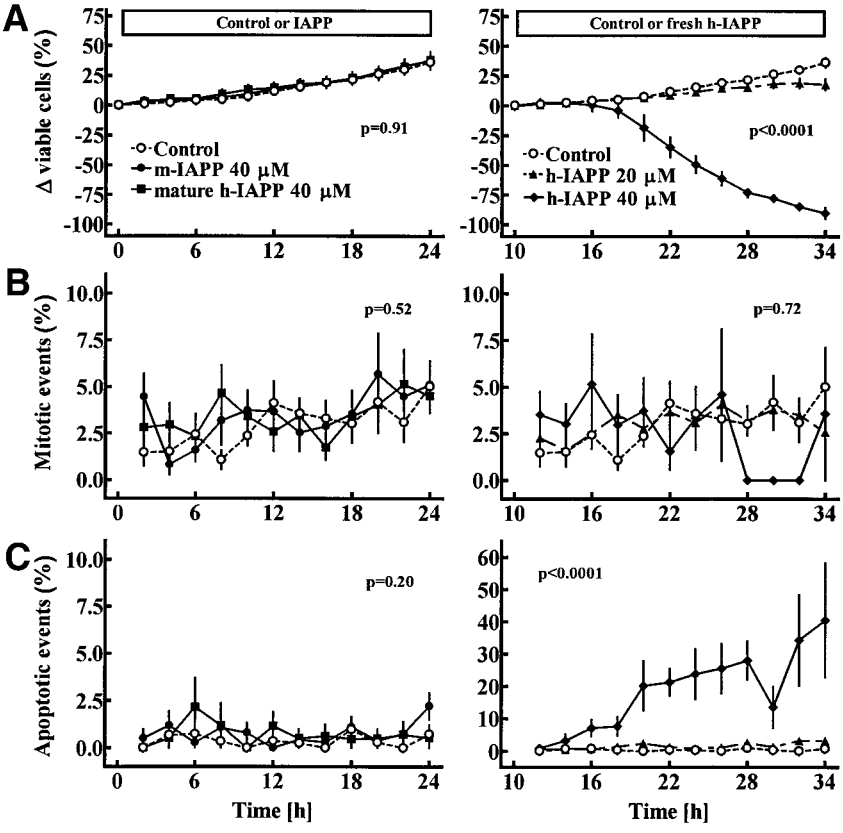

FIG. 2. Change in the relative size of the cell population (*A*), mitotic events (*B*), and apoptotic events (*C*) in RIN cells cultured for 24 h on the stage of a time-lapse video microscope. Control, *m*-IAPP, matured h-IAPP (*left*), or fresh h-IAPP (*right*) were applied to the cells in culture 10 h before initiation of time-lapse imaging ($P < 0.05$ indicates significant differences between groups; ANOVA).

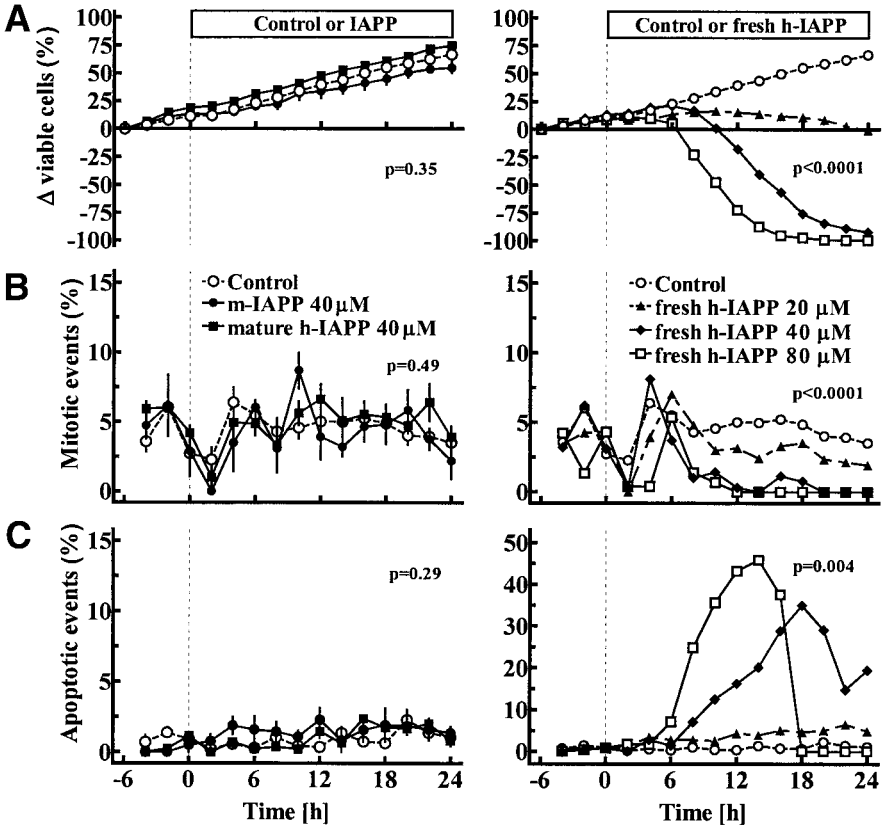

FIG. 3. Change in the relative size of the cell population (*A*), mitotic events (*B*), and apoptotic events (*C*) in HeLa cells cultured for 30 h on the stage of a time-lapse video microscope. After a basal period (−6 to 0 h), control, *m*-IAPP, matured h-IAPP (*left*), or fresh h-IAPP (*right*) were applied to the cells in culture ($P < 0.05$ indicates significant differences between groups; ANOVA).

μmol/l h-IAPP) and RIN cells (20 μmol/l h-IAPP), which previously underwent mitosis, were significantly more vulnerable to apoptosis than nondividing cells (Fig. 4). It is interesting that preferential apoptosis of replicating cells was also detected in control experiments without application of toxic IAPP aggregates in both cell types (Fig. 4). At high levels of h-IAPP–induced cytotoxicity, there was no preferential vulnerability of replicating cells to IAPP-induced apoptosis (Fig. 4). The frequency distribution of the time interval between apoptosis and previous replication in the same cell (Fig. 5) shows that >50% of death events in replicating β-cells occur within 3 h after replication. Compared with the full cell cycle length in RIN cells (26 ± 0.6 h), replicating cells were particularly vulnerable in the first ~12% of the cell cycle. HeLa cells were also characterized by a significant negative correlation between the relative frequency of apoptosis and the time interval after mitosis.

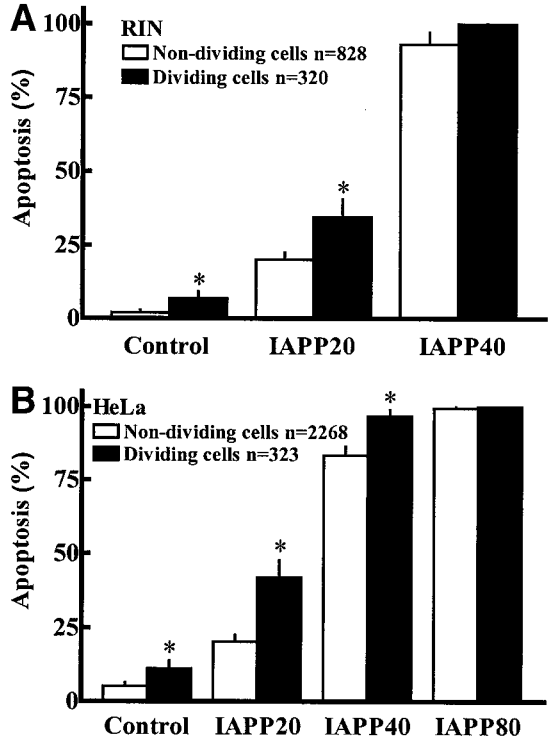

FIG. 4. Dose-response relationships of IAPP-induced apoptosis in dividing versus nondividing RIN (*A*) and HeLa cells (*B*). *Significant differences between both groups ($P < 0.05$, nonpaired *t* test).

Apoptosis in human islets exposed to h-IAPP. The number of apoptotic cells per islet was increased fivefold after 48 h of incubation with 40 μmol/l h-IAPP compared with control islets or incubation with *m*-IAPP (Fig. 6). Pairs of apoptotic cells were frequently observed at a comparable percentage of total apoptotic cells (P = NS) in all three groups: 41 ± 8% (control), 30 ± 7% (*m*-IAPP), and 44 ± 4% (h-IAPP).

Human autopsy pancreatic tissue. In the pancreas of humans with type 2 diabetes, the apoptotic cells in islets were frequently adjacent to each other or organized in pairs or doublets with two separate nuclei (Fig. 7), suggesting that cells underwent apoptosis shortly after mitosis. On average, 26 ± 5% of apoptotic cells in the islets of humans with type 2 diabetes were present in pairs. Paired cells may be present in any plane within the islet. Autopsy pancreatic tissue was studied in a single plane, whereas isolated islets exposed to h-IAPP were studied whole. Therefore, the observation that 26% of apoptotic cells in

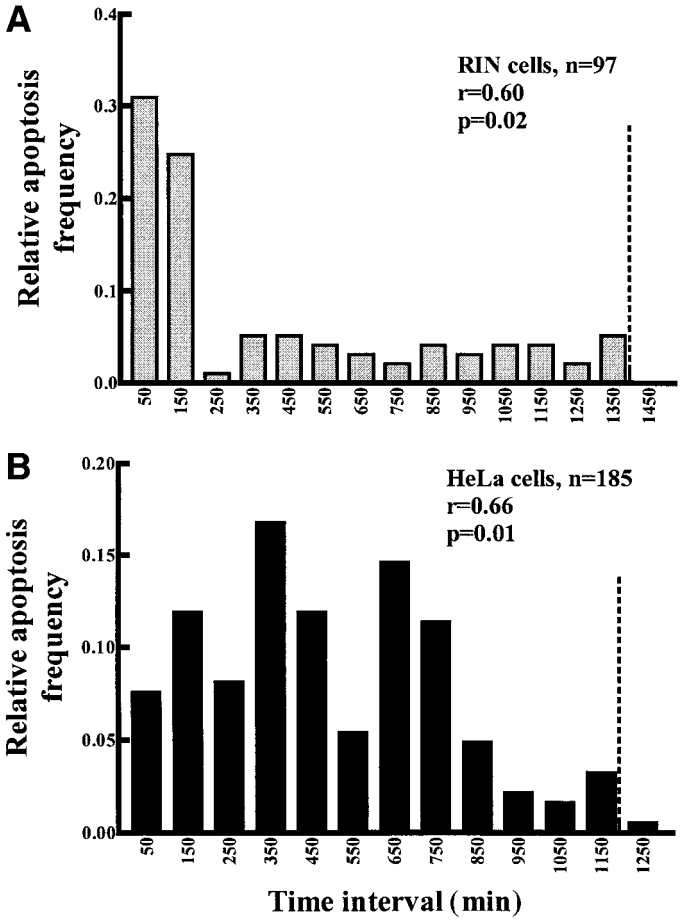

FIG. 5. Apoptosis in replicating RIN (*A*) and HeLa cells (*B*). Depicted are the histograms of the time intervals between replication and apoptosis in the same cell. The dashed vertical line indicates the full cell cycle length. The *r* and *P* values are derived from linear regression analysis.

autopsy pancreas are present as pairs is consistent with 44% of apoptotic cells present as pairs in isolated human islets.

DISCUSSION

In the present study, we sought to establish whether dividing cells are more vulnerable to h-IAPP–induced cytotoxicity and whether this cytotoxicity is mediated through apoptosis. These questions were addressed using human versus rodent IAPP applied to cell types with rapid rates of replication. These data were then supplemented by examining the pattern of cells undergoing apoptosis in human pancreatic tissue from patients with type 2 diabetes and in human islets incubated for 48 h with h-IAPP. Together, these studies support the concept that replicating β-cells are more vulnerable to apoptosis and that this might contribute to the deficit of β-cell mass in type 2 diabetes.

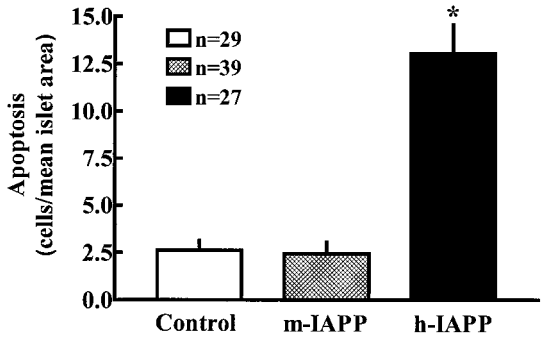

FIG. 6. Mean number of apoptotic cells in human islets incubated for 48 h with vehicle (control), *m*-IAPP (40 μmol/l), or h-IAPP (40 μmol/l). Apoptosis in human islets was detected using the TUNEL method. *Significant difference compared with control ($P < 0.05$, nonpaired *t* test).

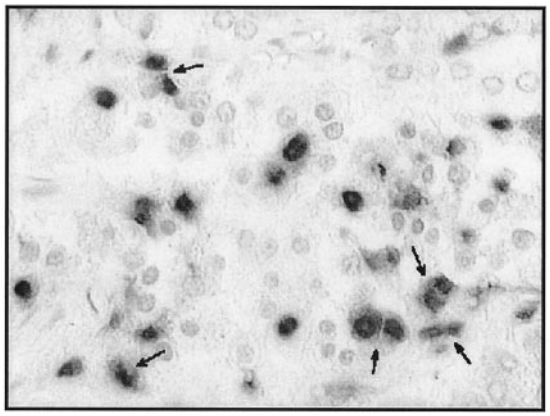

FIG. 7. Pancreatic tissue section from a human with type 2 diabetes stained for apoptosis using the TUNEL method. Arrows indicate pairs of apoptotic β-cells, suggesting postmitotic β-cell apoptosis. To illustrate the phenomenon of pairs of TUNEL-positive cells, the figure depicts an example with a high number of apoptotic cells. Recently, we reported that in autopsy material from humans with type 2 diabetes, the mean is 0.5 ± 0.2 apoptotic cells/islet ranging from 0 to 46 cells/islet (3).

The concept of a link between cell proliferation and apoptosis has evolved from the evidence of morphological (Fig. 1) and structural similarities between both processes (35,36). Also, mitosis has been recognized as a cell-cycle checkpoint during which the expression of apoptosis-inhibiting factors is induced (37) to counterbalance pro-apoptotic pathways that might induce apoptosis during mitosis (37). We now report experimental evidence to support the hypothesis that the rate of apoptosis in replicating rodent β-cells and human endothelial cells is increased, even under conditions without application of cytotoxic IAPP aggregates. This supports the notion that the link between cell proliferation and cell death is an inherent cell biological characteristic instead of a specific consequence of IAPP toxicity. If, in this setting, β-cells are exposed to low or medium levels of IAPP toxicity, then the rate of apoptosis will be higher in replicating β-cells, as shown by the present study. It also seems plausible that the cell-cycle–dependent susceptibility to apoptosis is overcome at very high levels of toxicity, which induce cell death in both dividing and nondividing cells.

Consistent with the results in cultured cells, we report that in the pancreas of humans with type 2 diabetes and in cultured human islets incubated with human IAPP, apoptotic β-cells are frequently organized in pairs. This pattern of apoptotic β-cells has recently been described in the pancreas and in isolated islets of a rodent animal model for diabetes (38) and was interpreted to suggest postmitotic β-cell apoptosis as a mechanism of β-cell death. The frequency of apoptotic cells in isolated human islets incubated even without h-IAPP (2.6 ± 0.5 cells per mean

islet area; Fig. 6) is increased compared with islets in whole pancreas (0.5 ± 0.2 cells per islet [3]). This observation presumably reflects loss of islet vasculature resulting in relative hypoxia and reduced supply with nutrients. Addition of h-IAPP increased the frequency of apoptotic cells in human islets fivefold. However, in islets cultured with or without h-IAPP, the proportion of apoptotic cells present in pairs was comparably high (~40%), again supporting the underlying premise that replicating cells have preferential vulnerability to apoptosis irrespective of the initiating factor.

In the present studies, we were able to directly visualize and time each cell death event. The results indicate that the mechanism of cell death was invariably apoptosis, which is consistent with the time between application of the freshly dispersed IAPP aggregates to the cells and the execution of cell death (~6–8 h for HeLa cells and 16–18 h for RIN cells). Interestingly, it has been reported that the time-course and the dose-response relationship for h-IAPP–induced apoptosis is similar in RIN cells compared with a human β-cell line (39).

In the present experiments, h-IAPP was applied to cells in culture at concentrations ranging from 20 to 80 μmol/l. The concentration of human IAPP in the immediate extracellular space surrounding β-cells is unknown, but presumably after β-cell stimulation, it may approach the estimated concentration present in β-cell vesicles (>20 μmol/l [40]). The concentration of IAPP applied in the present studies is therefore in the range likely to be locally present in islets in vivo. The current experiments were performed with extracellular application of toxic IAPP oligomers, whereas in vivo IAPP is expressed inside β-cells and trafficked to secretory granules. Previous studies have shown accelerated internalization of extracellular markers after exposure of cells to h-IAPP, consistent with accelerated endocytosis (41). In neuroblastoma cells, a 6-h incubation with Alzheimer's β1-42 protein induced increased lysosomal membrane permeability, which was interpreted as an indication for intracellular accumulation of the Aβ1-42 peptide (42). Therefore, extracellularly applied IAPP might act similar as endogenous IAPP to interact not only with the cell membrane but also with the membranes of intracellular organelles. In support of the rapid uptake of extracellular aggregates, we have reported the presence of numerous abnormal intracellular vesicles labeled for IAPP after application of freshly prepared h-IAPP (but not *m*-IAPP or matured solutions of h-IAPP) to cells examined subsequently by electron microscopy (25). It is therefore consistent that in transgenic mice expressing h-IAPP, studied prospectively during the time of development of diabetes, intracellular IAPP aggregates preceded development of extracellular IAPP amyloid and coincided with the maximal period of β-cell loss (17). Having noted that these small intracellular IAPP aggregates were invariably associated with disrupted cell membranes (17,43), we hypothesized that cell toxicity may be mediated by these deposits through membrane toxicity. This postulate was subsequently supported by studies showing that freshly dissolved h-IAPP caused membrane bilayers to leak (nonselective ion channels) (24) and caused cytotoxicity associated with abnormal cell membrane–associated vesicles, whereas matured amyloid fibrils from h-IAPP or rodent IAPP had no effect on membrane bilayers or cells (25). More recently, it has been reported that protofibrils of Alzheimer's β-amyloid protein promote the formation of membrane pores (26), supporting that inappropriate membrane permeabilization might be the cause of cell dysfunction or induction of cell death in amyloid diseases such as type 2 diabetes. Given these actions of IAPP aggregates on cell membranes, it is plausible that IAPP might induce apoptosis either by the mitochondrial pathway through disruption of mitochondrial membranes (cytochrome C leakage) or by prolonged elevation of intracellular calcium levels (cell membrane leakage), both of which are established pathways for the initiation of programmed cell death (44,45). Recently, one report suggested, however, that dysregulation of intracellular calcium concentrations might not be a trigger for h-IAPP–induced apoptosis in RIN cells (46). IAPP has also been reported to induce oxidative stress genes (cox-2 and IκB-α) and apoptotic genes (c-jun [39], junB, c-fos, fosB) when applied to rat cortical neurons (47).

In nondiabetic humans, IAPP is trafficked through the β-cell to insulin secretory vesicles from where it is cosecreted with insulin (10,40) without forming amyloid, despite its amyloidogenic propensity. Obesity, the main risk factor for development of type 2 diabetes in those genetically predisposed, causes a proportionally greater rate of expression of IAPP to insulin (14). However, most obese humans respond to obesity-induced insulin resistance by increasing β-cell mass (1,3,31) and trafficking and secreting the increased rates of IAPP expression without the development of islet amyloid or development of diabetes. We have postulated that people who are at risk for development of type 2 diabetes may have decreased capacity in one or more of chaperone proteins responsible for trafficking IAPP that, under conditions of insulin resistance, leads to the formation of toxic intracellular IAPP aggregates (48,49). Now we report that replicating β-cells are particularly vulnerable to these cytotoxic IAPP oligomers, and we postulate that this compromises the capacity of the endocrine pancreas to expand appropriately in the setting of insulin resistance in those genetically predisposed to form IAPP oligomers. Although the rate of β-cell replication is relatively low in humans (3,50), over a period of years, failed adaptive replication of β-cells would be expected to lead to a relative deficiency of β-cell mass, hyperglycemia, and type 2 diabetes. This model to account for the deficit in β-cell mass in type 2 diabetes is supported by prospective studies in obese mice transgenic for h-IAPP, which develop diabetes as a result of failure to expand rather than loss of β-cell mass. In these mice, the pattern of β-cell apoptosis mirrors that of the rate of β-cell replication (51).

Other causes of increased β-cell apoptosis in type 2 diabetes have been proposed, including toxicity arising from high concentrations of free fatty acids (52,53) and glucose (54). As we report here that increased vulnerability of recently replicating β-cells to apoptosis seems to be a fundamental property of β-cells (being present even in the absence of h-IAPP), we anticipate that this model is likely to be valid for any cause of increased β-cell apoptosis. Furthermore, because type 1 diabetes is believed to be due to increased β-cell apoptosis induced by autoimmune

cytokines, attempted regeneration of β-cell mass would also be prevented by apoptosis of replicating β-cells.

In summary, we report that replicating β-cells are more vulnerable to apoptosis induced by small oligomers of h-IAPP applied at concentrations inducing low levels of toxicity. These data are supported by the presence of paired apoptotic β-cells in human pancreatic tissue from cases of type 2 diabetes and isolated human islets exposed to h-IAPP, suggesting postmitotic apoptosis. We postulate that the β-cell deficit in type 2 diabetes is due to failure of β-cell expansion, rather than loss of β-cell mass, in response to insulin resistance. This model is consistent with recent studies that report prevention of type 2 diabetes by amelioration of insulin resistance by life style changes, metformin, or troglitazone.

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