Possible relationship between changes in islet neogenesis and islet neogenesis-associated protein-positive cell mass induced by sucrose administration to normal hamsters

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Abstract

The possible relationship between changes in islet cell mass and in islet neogenesis-associated protein (INGAP)-cell mass induced by sucrose administration to normal hamsters was investigated. Normal hamsters were given sucrose (10% in drinking water) for 5 (S8) or 21 (S24) weeks and compared with control (C) fed hamsters. Serum glucose and insulin levels were measured and quantitative immunocytochemistry of the endocrine pancreas was performed. Serum glucose levels were comparable among the groups, while insulin levels were higher in S hamsters. There was a significant increase in β-cell mass (P<0.02) and in β-cell 5-bromo-2’-deoxyuridine index (P<0.01), and a significant decrease in islet volume (P<0.01) only in S8 vs C8 hamsters. Cytokeratin (CK)-labelled cells were detected only in S8 hamsters. INGAP-positive cell mass was significantly larger only in S8 vs C8 hamsters. Endocrine INGAP-positive cells were located at the islet periphery (96%), spread within the exocrine pancreas (3%), and in ductal cells (<1%) in all groups. INGAP positivity and glucagon co-localization varied according to topographic location and type of treatment. In C8 hamsters, 49.1±6.9% cells were INGAP- and glucagon-positive in the islets, while this percentage decreased by almost half in endocrine extra-insular and ductal cells. In S8 animals, co-expression increased in endocrine extra-insular cells to 36.3±9.5%, with similar figures in the islets, decreasing to 19.7±6.9% in ductal cells. INGAP-positive cells located at the islet periphery also co-expressed CK. In conclusion, a significant increase of INGAP-positive cell mass was only observed at 8 weeks when neogenesis was present, suggesting that this peptide might participate in the control of islet neogenesis. Thus, INGAP could be a potentially useful tool to treat conditions in which there is a decrease in β-cell mass.

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Introduction

β-cell mass is the result of a final rate between opposed mechanisms such as growth and destruction. New β cells can be formed by either mitotic division of pre-existing differentiated β cells (replication), or differentiation from a developmental precursor or stem cell (neogenesis) (Bonner-Weir 1994, Bouwens & Klöppel 1996, Leahy 1996).

A wide variety of processes, such as partial pancreatectomy (Cardezza & Rodríguez 1949, Lee et al. 1989), infusion of glucose solutions (Bonner-Weir et al. 1989), dietary manipulation (Lombardo et al. 1983, Luo et al. 1998), normal pregnancy (Parsons et al. 1997), pancreatic duct ligation (Wang et al. 1995), wrapping of the pancreas head in cellophane (Rosenberg et al. 1983), and sulfonylurea administration (Loubatieres 1964), stimulate β-cell growth (Swenne 1982, Bonner-Weir et al. 1989, Bonner-Weir 1994). Understanding the precise mechanism underlying these changes – particularly those obtained by modifying food intake – becomes of great interest, especially within the context of prevention (Pan et al. 1997) and treatment (Salmerón et al. 1997) of type 2 diabetes.

Several genes, hormones and growth factors are involved in the control of this growth process (Nielsen & Serup 1998). One of these factors, islet neogenesis-associated protein (INGAP) has been identified as part of
a protein complex called ilotropin, isolated from pancreata of normal hamsters previously wrapped in cellophane (CW) (Pittenger et al. 1991). CW of the adult hamster pancreas led to the induction of new islet formations from ducts within 2 weeks with recapitulation of normal ontogeny (Bonner-Weir et al. 1993, Bonner-Weir & Smith 1994, Rosenberg et al. 1995, Leahy 1996) in the absence of an inflammatory response (Vinik et al. 1996). Thus, the appearance of INGAP is not the consequence of a previous acute pancreatitis, as is the case with the PAP (pancreatitis associated protein) and Reg/PSP families of genes (Gross et al. 1985, Giorgi et al. 1989, Terazono et al. 1990, Orelle et al. 1992).

It has recently been shown that INGAP is the product of a gene expressed in acinar cells but not in islets of regenerating hamster pancrea (Rafaello et al. 1997). Administration of ilotropin causes recapitulation of normal islet ontogeny and reverses streptozotocin (STZ)-induced diabetes mortality by 50% (Swenne 1982, Rosenberg 1985, Giorgi 1989, Leahy 1996). CW of the adult hamster pancreas, we recorded the total wet weight. Samples of the pancreatic tail were fixed in Bouin’s fluid and embedded in paraffin. Serial sections thinner than 5 μm were obtained from different levels of the block. After mounting each section from a given series on different slides, we stained adjacent sections for the immunocytochemical identification of β and non-β cells, cytokeratin- and 5-bromo-2’-deoxyuridine (BrdU)-labelled cells as well as INGAP-positive cells.

**Materials and Methods**

Twenty-four male Syrian hamsters weighing 30 ± 2 g (23 days of age) were maintained in a temperature-controlled room (23 °C) with a fixed 12-h light:darkness cycle (0600–1800 h). They were randomly divided into four groups: the two experimental groups had free access to a standard commercial diet plus 10% sucrose in the drinking water for 5 or 21 weeks (S8 and S24 respectively), whereas the two control groups received the same diet and tap water for the same periods (C8 and C24 respectively). Water intake was measured daily in all groups, while the body weight of each animal was recorded once a week throughout the experimental period.

**Blood parameters**

We measured glucose (glucose–oxidase method; Boehringer–Mannheim, Mannheim, Germany) and insulin (radioimmunoassay (Herbert et al. 1965)) levels in serum from each animal at the time of killing (around 0900 h).

**Morphological studies**

After dissecting fat tissue away from the intact excised pancreas, we recorded the total wet weight. Samples of the pancreatic tail were fixed in Bouin’s fluid and embedded in paraffin. Serial sections thinner than 5 μm were obtained from different levels of the block. After mounting each section from a given series on different slides, we stained adjacent sections for the immunocytochemical identification of β and non-β cells, cytokeratin- and 5-bromo-2’-deoxyuridine (BrdU)-labelled cells as well as INGAP-positive cells.

**Identification of islet β and non-β cells**

The following procedure was performed. Bouin-fixed, deparaffinized sections were treated with normal porcine serum to prevent non-specific staining and endogenous peroxidase was blocked with methanol/hydrogen peroxide; thereafter, the sections were incubated with appropriate dilutions of our own guinea-pig anti-insulin serum (1:20 000), plus a mixture of three other rabbit sera: antiglucagon (1:400), antipancreatic polypeptide (1:10 000) (both from Novo Nordisk A/G, Denmark), and antisomatostatin (1:6000) (Dako, Glostrup, Denmark).

**Antigen retrieval (cytokeratin (CK) immunostaining)**

Dепaraffinized sections were pretreated with 250 ml antigen-retrieval solution (Vector Laboratories, Burlingame, CA, USA) for 10 min in a microwave oven (500 W). To reveal the presence of CK-positive cells, we used a panspecific cocktail of antibodies (1:40) against human CKs (clone AE1–AE3; Dako).

**INGAP-reacting cells**

Cells were identified using a specific rabbit antibody (IgG1246, 1:600) against a synthetic pentadecapeptide corresponding to amino acids 20–39 of the deduced INGAP protein sequence. The antibody was provided by Eli Lilly and Company, Indianapolis, IN, USA.

**Islet cell replication rate**

Islet cell replication rate was estimated in pancreatic sections obtained from animals previously injected with 25 mg/100 g BrdU (Sigma Co., St Louis, MO, USA) i.p., 1 h before killing (Gratzner 1982). Sections were first incubated with BrdU–specific antibodies (1:100) (Cell Proliferation Kit-RPN 20; Amersham International plc, U.K.), and non-β cells, cytokeratin- and 5-bromo-2’-deoxyuridine (BrdU)-labelled cells as well as INGAP-positive cells.

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Amersham, Bucks, UK), and then the fraction of β, non-β, ductal and acinar cells was quantified among the labelled nuclei as described below. The replication rate – relation between the total number of cells counted (no less than 3000) and BrdU-labelled cells – was expressed as the percentage of BrdU-labelled cells from the total of such cells.

In all these cases, unless specified in the figure legends, we used the streptavidin–biotin complex method (ABC; Dako), and carbazole as chromophore. Additionally, we verified serologic specificity by preincubating a given antiserum with an excess of either the corresponding hormone or BrdU for 24 h at 4 °C, or by omitting the first peptide specific antibody in the immunostaining procedure.

**Double immunolabelling studies**

We followed two different procedures. (1) We performed double staining of the following pairs: β (insulin antibody) and non-β cells (pool of glucagon+somatostatin+ pancreatic polypeptide (PP) antibodies); β (insulin antibody) and BrdU (BrdU antibody); non-β (antibodies pool) and BrdU (BrdU antibody). We used the streptavidin–biotin complex method, with peroxidase and alkaline phosphatase together with carbazole and fast-blue, respectively, as chromogens. Incubations with primary antibodies were overnight whereas those with the secondary biotinylated antibodies were for 30 min. (2) For serial double-immunofluorescence labelling, sections pretreated with non-immune sera from both rabbit and mouse were mounted in Tris–glycerol (pH 8.4) for analysis by fluorescence microscopy.

**Morphometric analysis**

We performed the morphometric analysis by videomicroscopy in combination with OPTIMAS (Bioscan Incorporated, Edmons, WA, USA) software in order to estimate the following parameters: total pancreas area excluding connective tissue (TP); the areas of endocrine (En) and exocrine (Ex) pancreas and of β (βA) and non-β (nβA) cells; and islet volume (according to Hellman 1959). We also calculated volume density of endocrine pancreas (En/TP); and volume density of β (βA/TP) and non-β (nβA/TP) cells. To calculate the absolute β and non-β cell mass, the respective volume density was multiplied by the total weight of the pancreas (Bonner-Weir et al. 1989). A similar procedure was used to measure INGAP-positive cell mass and to define its distribution within pancreatic cells.

**Fluorescence optics**

A Zeiss Axiolab epifluorescence microscope equipped with an HBO 50 mercury lamp was used. For visualization of fluorescein labelling, we employed two different filters to detect rhodamine and fluorescein fluorescence.

For the quantitative evaluation of immunofluorescence, positively labelled endocrine cells were counted under a × 40 objective lens in sections made at different levels of the blocks. In each double-stained microscopic field, we counted, in succession, the cells that reacted positively for each one of the pairs of hormones tested, using first the appropriate set of filters for one of the fluorophores and then changing those filters to detect the other fluorescent probe. In doing so, we systematized our approach in such a way as to avoid scoring the same cell twice and thus were able to assess the total number of cells containing either or both of the hormones within each combination.

**Statistical analysis**

Results are expressed as the means ± s.e.m. The statistical significance was determined by Student’s t-test. Differences were considered significant when P<0.05.

**Results**

**Body weight and daily water intake**

No significant differences were recorded in the body weights of control and sucrose-fed hamsters either at 8 (C8, 68.3 ± 7.2 vs S8, 66.1 ± 8.6 g) or 24 (C24, 103.5 ± 5 vs S24, 116.28 ± 5 g) weeks. The S8 group drank a significantly larger volume of water than the C8 group (48.2 ± 2.3 vs 29.3 ± 4 ml; P<0.005). Similar differences were recorded in S24 and C24 animals (74.54 ± 1.3 vs 24.78 ± 0.98 ml; P<0.001).

**Quantitative and qualitative food ingestion**

The total weight and the caloric content of the daily food consumed per animal were equivalent between the
In all four groups, the pancreatic islets were composed of specifically stained endocrine cells, with β cells occupying the central zone and non-β cells located at the islet periphery. The islet shape ranged from round to ellipsoid, but the size was variable within a given histological section. A small number of ductal epithelial cells were positive for the insulin-specific antibody as well as for the pooled mixture of antisera against non-β cell types.

**Serum-glucose and insulin levels**

The glucose levels measured in all the animals throughout this study were within the concentration range of 128 ± 7 mg/dl found in our 20-week-old hamster colony (n=37) at the time of killing. In contrast, sucrose-fed hamsters had higher insulin levels: in S8 hamsters the increase was almost fourfold (2.29 ± 0.095 vs 0.62 ± 0.029 ng/ml; P<0.001), but the difference was smaller in S24 hamsters (2.15 ± 0.054 vs 1.38 ± 0.067 ng/ml; P<0.02). Due to the uneven behaviour of the blood glucose and insulin levels, great differences were recorded in the sucrose-fed groups relative to the corresponding control values in the insulin–glucose molar ratio (S8 vs C8, 5.3 × 10⁻⁹ vs 1.8 × 10⁻⁹; S24 vs C24, 5 × 10⁻⁹ vs 3.5 × 10⁻⁹).

**Morphological studies**

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**Morphometric studies**

There were no significant differences in C24 and S24 animals regarding the morphometric parameters, while the C8 and S8 hamsters differed significantly with respect to every parameter studied. The β-cell mass was significantly greater in S8 vs C8 (4.2 ± 0.2 vs 2.3 ± 0.5 mg; P<0.01), with similar values recorded in S24 and C24 animals (3.4 ± 0.3 vs 2.6 ± 0.4 mg). As previously reported (Del Zotto et al. 1999), significantly greater values were also recorded in S8 vs C8 in the number of islets per unit area (2.4 ± 0.1 vs 1.2 ± 0.1 mm²; P<0.004), and in β-cell size (97 ± 1.6 vs 88 ± 3.1 μm²; P<0.007). However, the islet volume in the S8 group was reduced to almost half that measured in C8 hamsters (38.9 ± 3.4 vs 66.8 ± 6.1 μm³, P<0.01). Similarly, the number of β cells/islet was also significantly smaller in S8 than in C8 animals (S8 vs C8, 54 ± 2 vs 87 ± 5.6; P<0.002).

The β-cell replication rate (BrdU incorporation) in S8 hamsters was tenfold that of C8 animals (P<0.007), but no significant differences were observed between S24 and C24 animals (Fig. 1). No significant differences were found in the replication rate of non-β cells within the islets between the S8 and the C8 groups (0.29 ± 0.11 vs 0.26 ± 0.14%; P=not significant (NS)). The BrdU–incorporation rate within the exocrine pancreas (ductal, acinar, and centroacinar cells) was comparable between
the S and the C groups (C8 vs S8, 3.3 ± 0.2 vs 3.6 ± 0.3, and C24 vs S24, 2.6 ± 0.2 vs 3.1 ± 0.4%).

As reported previously (Del Zotto et al. 1999), comparable values were recorded in non-β cell volume density, size, and mass in the control and in the sucrose-fed groups at any age (data not shown).

Presence of CKs in endocrine cells

Positive CK-stained cells, located at the islet periphery (in the α-cell locale) and in the ductal cells, were only found in S8 animals (Fig. 2), while no CK-positive cells were found in the islet periphery and in ductal cells in C8, C24 or S24 hamsters.

INGAP-positive cells

INGAP-positive cells were seen at the islet periphery (Fig. 3), within the extra-insular endocrine cells and in the ductus (Fig. 4). The mass of INGAP-positive cells was significantly larger in S8 than in C8 hamsters, while this difference disappeared in the mass of INGAP-positive cells in S24 animals (Table 1).

Most INGAP-positive cells in all groups were located at the islet periphery (control vs sucrose, 96.1 ± 0.7 vs 96.8 ± 3.8, and 97.3 ± 0.8 vs 97.7 ± 0.9 at 8 and 24 weeks respectively), with a minor proportion spread within the extra-insular endocrine tissue (3.1 ± 0.6 vs 2.6 ± 0.9 and 2.0 ± 0.8 vs 1.8 ± 0.5) and in ductal cells (0.8 ± 0.2 vs 0.6 ± 0.2 and 0.7 ± 0.3 vs 0.5 ± 0.1). No significant differences were recorded between the two experimental groups at any of the topographic distributions of INGAP-positive cells.

A certain proportion of INGAP-positive cells simultaneously reacted with the glucagon antibody, but not with the insulin, somatostatin or PP antibodies (Fig. 5). The frequency of glucagon co-expression in these cells varied according to their location and to the type of treatment received. In C8 hamsters, 49.1 ± 6.9% of the INGAP-positive cells were glucagon-positive in the islets, while this percentage value markedly decreased to almost half in INGAP-positive cells located at extra-insular or ductal level (Table 2). On the other hand, in the S8 animals the percentage of INGAP-positive cells that co-expressed glucagon increased in extra-insular endocrine cells to 36.3 ± 9.5% – with similar figures in the

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Table 1 INGAP-positive cell mass in 8- and 24-week-old control and sucrose-fed hamsters. Each value represents the mean ± S.E.M. of three animals in each age group

<table>
<thead>
<tr>
<th>Group</th>
<th>Islet-cell mass (mg)</th>
<th>Extra-insular-cell mass (mg)</th>
<th>Ductal-cell mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-week-old control</td>
<td>0.33 ± 0.003*</td>
<td>0.013 ± 0.003</td>
<td>0.002 ± 0.0005</td>
</tr>
<tr>
<td>8-week-old sucrose</td>
<td>1.70 ± 0.23†</td>
<td>0.026 ± 0.003†</td>
<td>0.008 ± 0.001†</td>
</tr>
<tr>
<td>24-week-old control</td>
<td>0.62 ± 0.08</td>
<td>0.012 ± 0.003</td>
<td>0.007 ± 0.002</td>
</tr>
<tr>
<td>24-week-old sucrose</td>
<td>0.62 ± 0.02</td>
<td>0.020 ± 0.004</td>
<td>0.005 ± 0.001</td>
</tr>
</tbody>
</table>

*P<0.01; †P<0.05; ‡P<0.02.

Table 2 Percentage of INGAP-positive cells co-expressing glucagon, with indicators of active neogenesis in 8-week-old sucrose-fed hamsters. Each value represents the mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>Islet (%)</th>
<th>Ducts (%)</th>
<th>Extra-insular cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>49.1 ± 6.9*</td>
<td>25.8 ± 8.0b</td>
<td>23.1 ± 9.9c</td>
</tr>
<tr>
<td>Sucrose</td>
<td>39.4 ± 4.8b</td>
<td>19.7 ± 6.9b</td>
<td>36.3 ± 9.5c</td>
</tr>
</tbody>
</table>

Cells counted were from pancreata of three animals from each group and three different levels each of the paraffin blocks. Despite the fact that the values recorded in each sector in the two experimental groups were different, such differences were not statistically significant. Conversely, significant differences were obtained within each group in the percentage of INGAP-positive cells according to their topographical distribution. Control: a vs b and a vs c, P<0.05; b vs c, NS; sucrose: a vs b and a vs c, NS; b vs c, P<0.05. The number of positive cells counted was as follows: control pancreas. Islet cells: INGAP, 1037; glucagon, 2107; INGAP and glucagon co-expression, 510. Ductal cells: INGAP, 51; glucagon, 73; INGAP and glucagon co-expression, 13. Extra-insular cells: INGAP, 41; glucagon, 195; INGAP and glucagon co-expression, 10. Sucrose-treated animals. Islet cells: INGAP, 945; glucagon, 2005; INGAP and glucagon co-expression, 373. Ductal cells: INGAP, 32; glucagon, 67; INGAP and glucagon co-expression, 6. Extrainsular cells: INGAP, 32; glucagon, 113; INGAP and glucagon co-expression, 12.

The current results confirm that after 5 weeks of sucrose feeding, the S8 hamsters showed an increase in total endocrine–pancreas mass stemming from islet neogenesis, β-cell hypertrophy and hyperplasia (Del Zotto et al. 1998, 1999). As previously shown, these changes were accompanied by a modest increment in insulin release at lower glucose concentrations (Del Zotto et al. 1998, 1999). The increased total endocrine pancreas and β-cell mass as well as the elevated β-cell mitotic rates seen in the S8 hamsters did not increase further in the S24 animals; rather, these parameters became similar to those found in controls. Moreover, no signs of islet neogenesis – predominance of small-sized islets and presence of CK–labelled islet cells – were observed in the S24 animals, thus indicating that islet neogenesis no longer occurred at this time-period (Del Zotto et al. 1998, 1999). CKs are expressed in the rat neonatal pancreas, where rapid pancreatic–islet growth occurs from the peri-islet mantle, but not within the region composed of mature endocrine cells (Bouwens et al. 1994, Wang et al. 1994). In our study, similar results were obtained in the pancreas of normal hamsters (Del Zotto et al. 1999); CKs are therefore good markers of the neogenic zone from which layers of mantle cells derived from the ductal epithelium are added to the islet mass.

We have previously shown (Massa et al. 1997), and later confirmed (Del Zotto et al. 1999), that the endocrine pancreas of the normal hamster attains adult morphological and secretory patterns long after birth. Such patterns present a different time-sequence: the number of islets and the β-cell mass reach adult characteristics at the age of 8 weeks with no further significant changes thereafter, while maximal glucose-induced insulin release is attained only at 24 weeks of age. This suggests that at each age, β cells can cope efficiently with the metabolic demands through different adaptive mechanisms: early in life, islet growth plays a predominant role, but since islet growth would be restricted at 24 weeks, at that time the tissue can respond to a sustained insulin demand through an enhanced capacity to release insulin in response to glucose.

It is generally accepted that, during fetal and neonatal life, islets are newly formed from precursor cells that bud off from the pancreatic ductules (Swenne 1982, Hellerström 1984). Islet growth occurs at their periphery (Conklin 1962), as observed in our S8 hamsters, where cells appear simultaneously labelled with glucagon and CK antibodies, the latter being useful markers to identify epithelial cell differentiation. As also found by other authors, only non-β cells showed immunoreactivity for CKs in the islets, suggesting that these cells are likely to harbour the proto-differentiated stem cells (Bouwens et al. 1994, Wang et al. 1994). Our results therefore suggest that sucrose feeding duplicates fetal pancreatic development: islet cells originate within a peripheral neogenetic zone.
indicating that they not only bud off from the ducts, but
their volume can further increase by adding consecutive
layers of a CK-positive cell mantle derived from the ductal
epithelium.

An increased INGAP-positive cell mass was only
observed in S8 hamsters, coinciding with clear signs of
islet neogenesis. Further, there were no changes in this
parameter in S24 hamsters when signs of neogenesis
were no longer detected. The INGAP-positive cells
were mainly located at the islet periphery, and they
never showed a positive reaction with the BrdU
antibody, but frequently did with the CK and glucagon
antibodies. It is therefore tempting to speculate that
they just added at the islet periphery after budding off
from the ducts, and that INGAP-positive cells with
no glucagon co-expression might still be at a less
derifferentiated stage.

The other INGAP-positive endocrine cells
co-expressing glucagon found at extra-insular level – also
depicted only in S8 animals – might correspond to
peripheral sections of the small-sized islets, which are
characteristic of this experimental group. On the other
hand, endocrine cells spread within the acinar tissue,
reacting with the INGAP antibody but not with the
glucagon antibody, might be cells that express the
INGAP gene and have not yet differentiated into a
specific islet hormone, or present hormone levels below
the sensitivity of the detection method employed
(Rafaeloff et al. 1997).

Based on the significant increase in the INGAP-positive
ductal-cell mass observed in the S8 hamsters, we expected
to find an increased number of these cells labelled with
BrdU. The absence of such a finding might be the
consequence of a rather short S-phase of ductal compared
with islet cells, which could handicap the chances of
labelling when using the time-schedule suitable for islet
cells. It might thus be important to sample tissues at earlier
time-points.

We have previously reported that sustained sucrose
feeding of normal rats (Lombardo et al. 1996) and hamsters
(Del Zotto et al. 1998, 1999) caused morphometric
changes – indicative of islet neogenesis – similar to those
currently described, while Rosenberg et al. (1988) ob-
served that CW of adult hamster pancreata produced a
2.5-fold increase in islet-cell mass as a consequence of islet
neogenesis. There were, however, some differences
between CW (Pittenger et al. 1991) and our model: while
in their control hamsters there was no INGAP expression
and it appeared only after CW, INGAP-positive cells
were already present in our control animals and their
numbers increased significantly only when clear signs of
neogenesis were present, i.e. in S8 hamsters. Differences
in behaviour could probably be ascribed to the different
strains employed by the two groups. For example,
our hamsters have lower mean glucose levels –
around 130 mg/dl – compared with the 200 mg/dl in
the Canadian Norfolk strain. The identification of
INGAP-expressing cells in normal baboon fetuses and
mouse embryos (Rafaeloff et al. 1998) also supports this
assumption. Further differences were found between
these two models: no apparent changes in peripheral
insulin levels were reported in CW animals (Rosenberg &
Vinik 1989), while our sucrose-treated hamsters exhibited
elevated insulin levels together with normoglycaemia
and a high insulin:glucose molar ratio, presumably reflect-
ing a degree of insulin resistance (Del Zotto et al. 1998,
1999).

It has been shown that the combination of high serum
insulin levels together with a high insulin:glucose molar
ratio, as is the case in our sucrose-fed hamsters, represents
a pancreatic compensatory response to insulin resistance
(Reaven 1988, De Fronzo 1997). Thus, the observed
changes in the β-cell mass (increased β-cell size,
replication rate, and islet neogenesis) of S8 hamsters
might represent a pancreatic compensatory response to
cope with the increased insulin demand. Although there is
a clear overlap between the changes recorded in INGAP-
positive cell mass and β-cell mass, it is, however, not
completely clear which role might play the former in the
mechanism of islet response, particularly in its neogenetic
component.

There are facts other than these currently described
suggesting that INGAP is involved in the process of islet
neogenesis. (1) Administration of a partially purified pro-
tein extract isolated from CW pancreata, called ilotropin,
causEd recapitulation of normal islet ontogeny and reversed
STZ diabetes mortality by 50% (Gross et al. 1985,
Pittenger et al. 1991). (2) A synthetic pentadecapeptide,
corresponding to a region unique to INGAP, significantly
stimulated thymidine incorporation into the hamster duct
epithelium in primary culture and into a rat pancreatic
duct cell line, having no effect on a hamster insulinoma
tumour cell line (Rafaeloff et al. 1997). (3) INGAP is
the product of a gene expressed in the regenerating hamster
pancreas (Rafaeloff et al. 1997). Western blot analysis
demonstrated the presence of INGAP in ilotropin but not
in extracts from control pancreata. Finally, (4) ilotropin
induced islet neogenesis by a paracrine or autocrine
mechanism rather than by a hormonal one (Rosenberg
et al. 1983, 1996). Taken together, the available evidence
suggests that the INGAP gene is a pancreatic gene whose
expression is either triggered (CW hamsters) or enhanced
(sucrose-fed hamsters) during islet neogenesis, and that its
protein product – a constituent of ilotropin (Pittenger et al.
1991) – is capable of initiating duct cell proliferation and
derifferentiation.

In our sucrose-fed model, INGAP might be the link
between the increased insulin demand and pancreatic
changes, playing an important regulatory role in islet
growth and neogenesis. The present results were obtained
with a relatively short-term dietary manipulation, lending
further support to the concept that INGAP is a key
component in the process of islet neogenesis, and might therefore be considered a potential tool for the treatment of conditions in which β-cell mass is seriously decreased or damaged.

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