Abstract  Functional heterogeneity among pancreatic beta cells is a characteristic feature of the islets of Langerhans. Under physiological conditions, beta cells in the pancreas of fed rats exhibited heterogeneous immunohistochemical staining for insulin and glucokinase. Intracellular beta cell glucokinase staining was either faint or dense. In the pericapillary space beta cell glucokinase immunoreactivity had a polar orientation, with the highest density in cytoplasmic regions close to the blood vessels. Starvation resulted in a loss of heterogeneity with homogeneous insulin staining in all beta cells of the islets, and this was accompanied by a loss of heterogeneous glucokinase staining. The intracellular polarity of glucokinase staining in contact to blood vessels also disappeared after starvation. Refeeding resulted in the reappearance of intercellular heterogeneity. In dependence on the functional demand, the endocrine pancreas recruited insulin from beta cells according to a well-defined hierarchy, with an initial preferential mobilization of medullary beta cells. In the course of this process intracellular polarity of glucokinase staining reappeared in areas of the beta cell with functional contact to the GLUT2 glucose transporter in the plasma membrane. This can be regarded as the morphological correlate of an activation of the glucose signal recognition apparatus. Interestingly, the study also provides evidence that the changes in glucokinase distribution apparently preceded those in insulin distribution, which is in keeping with the central role of glucokinase as the glucose sensor of the pancreatic beta cell.

Key words  Rat · Pancreatic beta cell · Insulin · GLUT2 glucose transporter · Glucokinase

Introduction

Insulin is distributed heterogeneously in pancreatic beta cells, and this heterogeneous distribution correlates with differences in the functional activity of the beta cells in the islets of Langerhans [9, 22, 25]. However, the underlying reasons for this heterogeneity are unknown. From in vitro studies on single beta cells there is evidence that the metabolic state of the individual beta cell determines its insulin secretory activity [5]. Glucokinase, the low-affinity glucose-phosphorylating enzyme, has a pivotal role in the pancreatic beta cell as the so-called glucose sensor [18] or glucose recognition enzyme [11] for initiation of glucose-induced insulin secretion. Together with the GLUT2 glucose transporter, glucokinase couples changes in the millimolar glucose concentration range to corresponding changes of the metabolic flux rate in pancreatic beta cells, and finally to the rate of insulin secretion [12, 19]. Colocalization studies have shown that the GLUT2 glucose transporter is mainly expressed in the plasma membrane of the pancreatic beta cell [21, 26] while glucokinase is localized in the cytoplasm [8, 20]. The distribution pattern of glucokinase is heterogeneous in the islets of Langerhans. It is not known, however, whether changes in the distribution of glucokinase participate in the nutrient-dependent regulation of this enzyme with concomitant effects on the secretory activity of the pancreatic beta cell.

In the present study we have adopted an immunohistochemical approach using semithin sections of plastic-embedded rat pancreas [4, 9], together with a computer-assisted quantification system. With this method we have studied the effects of starvation and refeeding on the pattern of glucokinase distribution and compared it with the distribution of insulin and GLUT2 immunoreactivity in the pancreatic islet beta cells.

Materials and methods

Pancreatic tissue was obtained from 3- to 4-month-old male Wistar rats. Four fed rats kept under normal laboratory conditions (blood
glucose: 5.8±0.3 mM) served as controls. Four rats were fasted for 48 h (blood glucose: 5.8±0.2 mM), and four additional fasted rats were subcutaneously injected for 24 h (blood glucose: 5.9±0.3 mM).

Small tissue specimens from the spleenic, gastric and duodenal parts of the pancreas were quenched in isopentane, precooled in liquid nitrogen, freeze-dried (−35°C for 72 h) and fixed by vapor-phase p-formaldehyde as described in detail elsewhere [9]. Freeze-dried specimens were used for analysis of immunoreactivities of insulin, GLUT2 glucose transporter and glucokinase [10]. The smaller specimens were embedded in Araldite.

Polyclonal antisera against insulin (Novo, Bagsvaerd, Denmark), diluted 1:7,000, and against the rat GLUT2 glucose transporter (WAK-Chemie, Bad Homburg, Germany), diluted 1:20,000–1:30,000, were used. For immunohistochemical staining of glucokinase we used a polyclonal, affinity-purified, antibody raised in rabbits in our laboratory against rat recombinant liver glucokinase at a dilution of 1:25–1:50. Western blot analyses of cytoplasmic fractions from rat islet and rat liver revealed a single 56-kDa band typical for glucokinase with no cross-reactivity to hexokinase.

The antisera against insulin and GLUT2 have been examined for method and antibody specificities [9, 10, 26]. In the present study the antisera against rat GLUT2 glucose transporter was tested by pre-adsorption with the GLUT2 glucose transporter peptide (WAK-Chemie, Bad Homburg, Germany) and peptides with unrelated specificities (insulin and glucagon at concentrations between 6.25 and 100 µg/ml). Upon the pre-adsorption with the homologous protein the cytoplasmic immunostaining for glucokinase in pancreatic beta cells was already absent.

The antisera against rat glucokinase was pre-adsorbed by affinity-purified glucokinase protein at concentrations as low as 6.25 µg/ml, the GLUT2 glucose transporter immunoreactivity in pancreatic beta cell plasma membrane and cytoplasm disappeared completely. The antisera against rat glucokinase was pre-adsorbed by affinity-purified glucokinase protein at concentrations between 5 and 50 µg/ml. After pre-adsorption with even the lowest concentration of the homologous protein the cytoplasmic immunostaining for glucokinase in pancreatic beta cells was already absent.

Serial semithin sections (0.5 µm) were immunostained by the avidin–biotin complex (ABC) method [6]. Following the fast removal of the resin and overnight incubation with the first antibody, biotinylated goat anti-rabbit IgG (1:100, 30 min) and a streptavidin–biotin–peroxidase complex (1:1,000, 30 min) were used as second and third antibodies (both from Jackson Immuno Research, West Grove, Ill.). The demonstration of the peroxidase was performed with 0.7 mM diaminobenzidine and 0.002% H2O2 in 0.05 mM Tris HCl buffer, pH 7.6.

A total of 1,700 sections studied was composed of smaller series of 20–40 semithin sections from different pancreatic regions of each experimental group and of five series of 100 sections. The smaller series were immunostained sequentially for insulin, GLUT2 glucose transporter and glucokinase. To demonstrate the heterogeneous intracellular distribution of glucokinase, the larger series were also monovalent immunostained for glucokinase twice in the fed control pancreas and once under each of the three other experimental conditions. The sections were viewed by bright field illumination or phase contrast with a Zeiss Photomicroscope II (Zeiss, Oberkochen, Germany).

In order to verify the changes in the immunoreactivities of insulin and glucokinase under fasting and refeeding conditions compared with the control developed in parallel, both immunoreactivities were densitometrically determined using a computer-assisted method. The image analysis system consisted of a Zeiss Photomicroscope II, a Sony CCD DXC-151 AP color video camera, and an Apple Macintosh Power PC 7500 equipped with a frame grabber card. The images of the islets made with a constant illumination value were import-ed into the system, displayed at a final magnification of 1:400 on a monitor and processed by the NIH shareware Image Analysis Program (Version 1.59) as described in detail by Russ [23]. First the beta cell area of each islet was determined. The screen resolution for the displayed digitized images was 1280×1024 pixels 8 bit monochrome/256 grey levels as arbitrary units (1=white; 256=black). After an automatic background subtraction (2 D roller ball) of the islet images the grey values of the immunoreactivities ranged between 8 and 157 for insulin and between 8 and 114 for glucokinase. The highest density in endocrine cells without immunostaining was 15 in the nuclear areas. The number of pixels was multiplied by the concomitant grey value to achieve the weighting of a particular immunoreactivity. The sum of all weighted pixels was divided by the measured beta cell area to obtain the integrated density per square micrometer of beta cell area. The results are presented as means±SEM and were tested for statistical significance with Student’s t-test.

Results

Beta cells in the fed control rat pancreas

Irrespective of the pancreatic region, pancreatic beta cells contained insulin immunoreactivity with variable density (Fig. 1a). Pancreatic beta cells in large islets (>150 µm diameter) exhibited heterogeneous staining for insulin. Beta cells in the cortex region in the periphery, which have contacts both with beta cells and with other endocrine cells, such as α- and δ-cells, expressed mostly dense insulin immunoreactivity, while medullary beta cells in the centre of the islet were faintly stained for insulin (Fig. 1a). Pancreatic beta cells in smaller islets (Fig. 2a) and single beta cells at extra-islet sites (Fig. 3a) displayed mostly dense insulin immunoreactivity. A quantitative analysis of the integrated density of insulin immunoreactivity expressed per square micrometer of islet area revealed a significant decrease in density with increasing islet size (Fig. 4).

Pancreatic beta cells in large islets (>150 µm diameter) displayed cytoplasmic staining for glucokinase with a heterogeneous distribution pattern (Fig. 1c). The intracellular glucokinase immunoreactivity in individual beta cells was homogeneously distributed in the cytoplasm with faint or dense glucokinase immunostaining in the cytoplasm of beta cells.

Fig. 1a–c Medium-sized pancreatic islet of a fed control rat. Semithin sections immunostained for a insulin, b GLUT2 glucose transporter and c glucokinase. Cortical beta cells (arrows: dense) and medullary beta cells (asterisks: faint) are heterogeneously immunostained for insulin in the same islet. The GLUT2 glucose transporter is restricted to the plasma membrane with gaps in close relation to the intra-islet capillary system (arrows). The glucokinase immunoreactivity is localized in the cytoplasm of beta cells with either homogeneously distributed faint (asterisks) or dense (arrows) immunostaining or immunostaining with polar orientation (arrowheads). ×425 d Glucokinase distribution of a fed control rat at a higher magnification. Pancreatic beta cells show either homogeneous dense (arrows) or faint (asterisks) immunostaining or immunostaining with polar orientation (arrowhead). Beta cells marked with asterisks are separated by a capillary. In both beta cells a denser glucokinase immunoreactivity is found under the plasma membrane in close association with the pericapillary space. ×650

Fig. 2a–c Two small pancreatic islets of a fed control rat. Semithin sections immunostained for a insulin, b GLUT 2 glucose transporter and c glucokinase. Cortical beta cells (arrows: dense) and medullary beta cells (asterisks: faint) are densely immunostained for insulin in the same islet. The GLUT2 glucose transporter is restricted to the plasma membrane with gaps in close relation to the intra-islet capillary system (arrows). The glucokinase immunoreactivity is localized in the cytoplasm of beta cells with either homogeneously distributed faint (asterisks) or dense (arrows) immunostaining or immunostaining with polar orientation (arrowheads). ×425 d Glucokinase distribution of a fed control rat at a higher magnification. Pancreatic beta cells show either homogeneous dense (arrows) or faint (asterisks) immunostaining or immunostaining with polar orientation (arrowhead). Beta cells marked with asterisks are separated by a capillary. In both beta cells a denser glucokinase immunoreactivity is found under the plasma membrane in close association with the pericapillary space. ×375

Fig. 3a–c A single pancreatic beta cell at an extra-islet site of a fed control rat. Semithin sections immunostained for a insulin, b GLUT2 glucose transporter and c glucokinase. This beta cell with a clear association to a capillary (asterisk) exhibits dense insulin and glucokinase immunoreactivity. The GLUT2 glucose transporter immunoreactivity is localized mainly in the plasma membrane, and the dense glucokinase is found in the cytoplasm of beta cells without intercellular variations. ×800