Glucokinase is located in secretory granules of pancreatic D-cells

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Abstract We immunohistochemically examined the distribution of glucokinase in rat pancreatic islets. Glucokinase immunoreactivity under light microscopy was detected in the cytoplasm of somatostatin cells as well as in that of insulin cells. No specific immunoreactivity was detected in glucagon and pancreatic polypeptide cells. In somatostatin cells, glucokinase immunoreactivity was located by electron microscopy exclusively within secretory granules.

Key words: Glucokinase; Pancreatic islet of Langerhans; Somatostatin; Secretory granule; Diabetes mellitus

1. Introduction

Glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) is often called hexokinase IV and catalyzes the phosphorylation of glucose. It is distinguished from other hexokinase isozymes (types I–III) by its low affinity for glucose and lack of inhibition by glucose 6-phosphate [1]. This enzyme has been identified only in pancreatic islet cells, hepatocytes, and some neuroendocrine cells [2]. Expression of glucokinase in pancreatic B-cells [3,4] and hepatocytes [1] is believed to be of major physiological importance for the maintenance of glucose homeostasis. It has been established that glucokinase in B-cells of pancreatic islets acts as a glucose sensor for glucose-induced insulin secretion [5,6]. However, the distribution of the enzyme in pancreatic islets has not yet been fully examined.

Similarities between the effects of glucose on somatostatin and insulin secretion were shown with respect to various points, i.e. sigmoidal dose-response relationship [7], glucose-anomeric preference [8], and inhibition by mannoheptulose, an inhibitor of glucokinase [8]. Both the sigmoidal dose-response curve and the α-anomeric preference in glucose-induced insulin secretion were explained by the kinetic properties of the glucokinase reaction [6,9,10]. Therefore, it is to be expected that glucokinase exists in D-cells as well as in B-cells and serves as a glucose sensor in the former cells.

The aim of the present study was to examine the glucokinase distribution in pancreatic islet cells and to obtain more information on the physiological function of glucokinase in this organ. This is the first report on the ultrastructural examination of glucokinase distribution.

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2. Materials and methods

2.1. Antibody

The antibody against glucokinase was generated in a rabbit that had been immunized with an 11-amino acid peptide with the sequence (NH_2)-YACKKACMLAQ-(COOH). For preparation of the anti-peptide antibody, 100 μg of the synthetic peptide coupled to keyhole limpet hemocyanin in Freund's complete adjuvant was injected subcutaneously into a New Zealand white rabbit, and booster injections were given once a month for 4 months.

2.2. Affinity purification of the anti-peptide antibody

One milliliter of antiserum against anti-peptide was loaded onto a 1-ml HiTrap protein A column (Pharmacia, Sweden). After the column had been washed with 0.1 M Tris-HCl buffer (pH 8.0), the bound IgG was eluted from the column with 0.1 M glycine-HCl buffer (pH 2.8). Fractions (3 ml each) were collected into tubes containing 0.5 ml of 1 M Tris-HCl buffer (pH 8.0). The IgG was further purified by sequential adsorption to and elution from rat liver glucokinase immobilized onto poly(vinylidene difluoride) membranes as described previously [11].

2.3. Immunohistochemistry

2.3.1. Fixation. Male Wistar rats weighing 180–220 g were used. They had free access to water and food (MM-3; Funabashi Farm, Japan). The animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and perfused through the left ventricle with 0.15 M phosphate-buffered saline (PBS) (pH 7.3) and successively with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The pancreas was then removed and minced into small tissue blocks. The blocks were further immersed in the same fixative for 12 h at room temperature, dehydrated through a graded ethanol series and propylene oxide, and embedded in Epon 812 [12].

2.3.2. Light-microscopic immunohistochemistry. Semithin sections were cut at a thickness of 0.5 μm on an LKB microtome (Ultrotome V), and mounted on glass slides by heating (80°C). Resin was removed from the sections with sodium methoxide according to the method of Mayor et al. [13] immediately before immunostaining. The sections were incubated consecutively with affinity-purified anti-peptide IgG (diluted 1:10 in 5% normal goat serum) overnight at 4°C, with biotin-labeled goat serum against rabbit IgG (Seikagaku, Japan) for 1 h at room temperature, and with streptavidin-peroxidase (Seikagaku, Japan) for 30 min at room temperature. Each incubation was followed by three washes with PBS. Finally, the sections were soaked for 15 min at room temperature in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.0125% 3,3′-diaminobenzidine·4HCl and 0.01% (w/v) H_2O_2, washed with distilled water, dehydrated, mounted with Entellan New (Merck, Germany), and examined by light microscopy.

2.3.3. Electron-microscopic immunocytochemistry. A post-embedding, immunogold-staining method was adopted. Ultrathin sections were cut on an LKB microtome and mounted on non-coated nickel grids. After having been etched with a saturated aqueous solution of sodium metaperiodate for 1 h, the grids with sections were incubated overnight at 4°C with anti-peptide antibody (diluted 1:10). The grids were then incubated with anti-rabbit IgG coupled to 5-nm gold particles (AuroProbe EM GAR G5, Amersham, UK). After completion of the immunostaining procedures, the grids were treated with a silver-enhancement reagent (IntenSE M Kit, Amersham, UK) to increase the size of the gold marker. The specimens thus prepared

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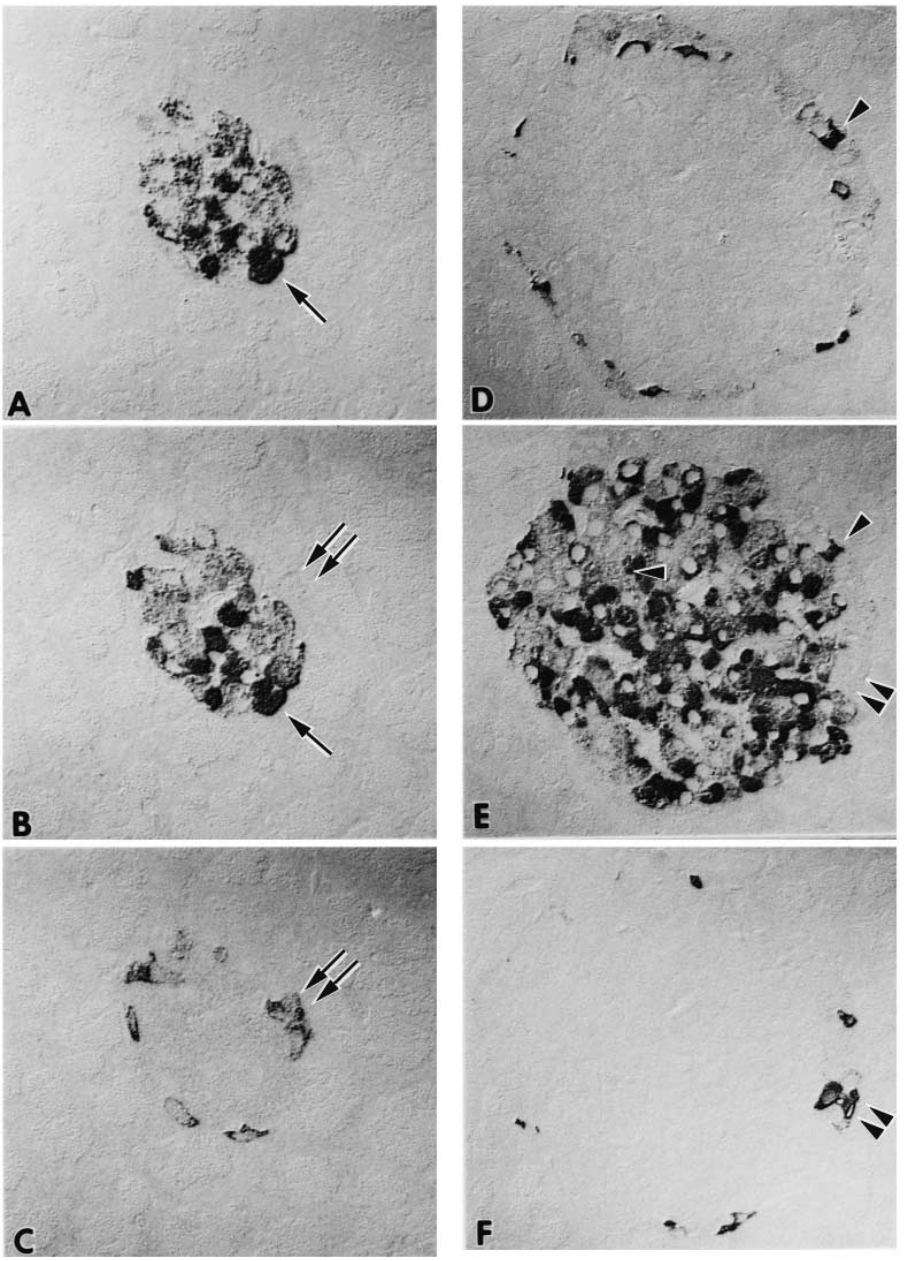

Fig. 1. Two series (A–C and D–F) of serial semithin sections showing immunohistochemical distribution of glucokinase (B, E), insulin (A), glucagon (C), somatostatin (D), and pancreatic polypeptide (F) in pancreatic islets. Arrow, double arrow, arrowhead, and double arrowhead indicate insulin-, glucagon-, somatostatin-, and pancreatic polypeptide-containing cells, respectively. Bar = 25 μm.

were stained with uranyl acetate and examined with a JEOL 1200EX transmission electron microscope under an accelerating voltage of 80 kV.

2.4. Specificity controls

In control experiments, semithin sections were incubated with anti-peptide antibody that had been absorbed with an excess of purified rat liver glucokinase, with non-immune rabbit IgG, or with pre-immune rabbit IgG, followed by consecutive incubation with biotin-labeled goat serum against rabbit IgG and streptavidin-peroxidase. Some sections were directly incubated with the second antibody without any preceding incubation with anti-peptide antibody.

3. Results and discussion

Clear and positive glucokinase immunoreactivity was observed in pancreatic islets by light microscopy (Fig. 1B,E). We established the specificity of immunostaining for glucokinase as follows: (i) no staining was observed when non-immune or pre-immune IgG was used instead of the anti-peptide antibody, (ii) no staining was observed when sections were incubated with the second antibody without preceding incubation with the anti-peptide antibody, (iii) immunoabsorption of the anti-peptide antibody with purified rat liver glucokinase completely prevented immunostaining of glucokinase, and (iv) no staining was seen in the sections of kidney and skeletal muscle of rats incubated with anti-peptide antibody (data not shown).

Glucokinase immunoreactivity was restricted to the islets of Langerhans. Analysis of consecutive sections immunostained for insulin, glucagon, somatostatin, and pancreatic polypeptide indicated that glucokinase was present in B-cells

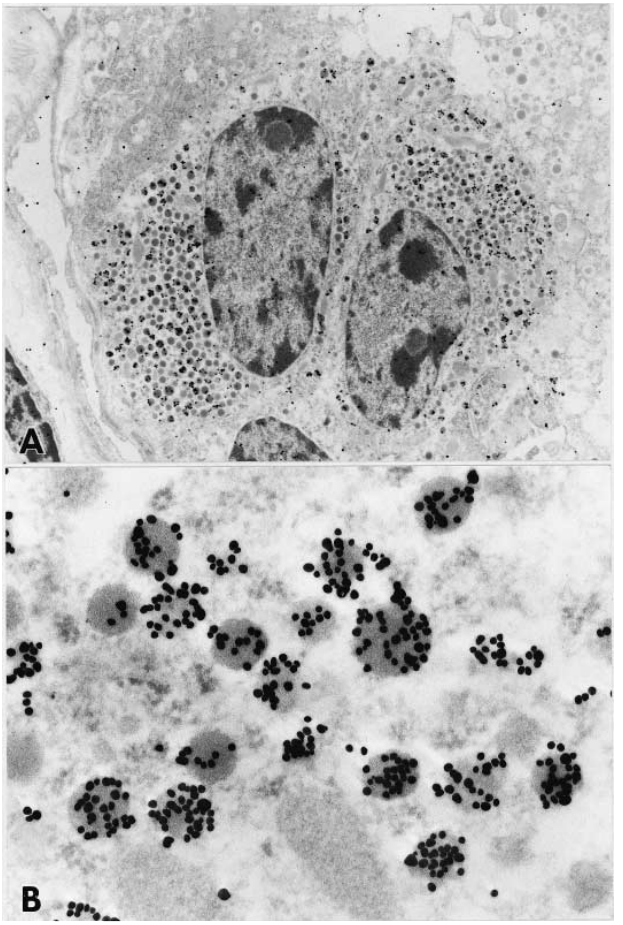

Fig. 2. Ultrastructural distribution of glucokinase in D-cells examined by the immunogold method. The location of glucokinase immunoreactivity within somatostatin-secretory granules is shown at low (A) and high (B) magnification. Original magnification: (A) ×10 000; (B) ×76 000.

(Fig. 1A,B) and D-cells (Fig. 1D,E), but not in A-cells (Fig. 1B,C) or PP-cells (Fig. 1E,F). In both B- and D-cells, glucokinase immunoreactivity was present predominantly in the cytoplasm. In addition, it should be noted that the extent of glucokinase immunoreactivity in B- and D-cells was positively correlated with that of immunoreactivity of insulin and somatostatin, respectively, indicating that the majority of glucokinase in pancreatic islets is located in the cytoplasm of B- and D-cells. The absence of glucokinase immunoreactivity in A-cells was contradictory to a previous paper [14] reporting that rat pancreatic A-cells express glucokinase mRNA and protein. One of the possible explanations for this contradiction is that the amount of glucokinase in A-cells is too low to be detected by our immunohistochemical method.

At the electron microscopic level, glucokinase immunoreactivity in D-cells was present exclusively within somatostatin-secretory granules (Fig. 2). Glucokinase immunoreactivity as assessed by light microscopy was somewhat higher in D-cells than in B-cells (Fig. 1), suggesting that the intracellular concentration of the enzyme is higher in the former cells than in the latter ones. This might be the reason for the clear staining of glucokinase in D-cells and for the subtle staining in B-cells observable by electron microscopy.

It is not yet known whether the glycolytic pathway operates in somatostatin-secretory granules. If it is the case, it is likely that glucokinase in the granules acts as a glucose sensor as demonstrated in B-cells. If the enzymes of the glycolytic pathway are not present all together in the granules, an unknown function of glucokinase should be explored. Very recently, nitric-oxide synthase was reported to be present in the secretory granules of pancreatic A- and D-cells [15]. Some investigators also reported that a type II isoform of adenylate cyclase was located in the secretory granules of pancreatic A- and B-cells [16]. The function of the two enzymes in the secretory granules has not yet been defined. Elucidation of the physiological meaning of the presence of these enzymes including glucokinase should shed light on the mechanism of hormone release from the pancreatic islet.

Noma et al. [17] indicated that the stimulation of islet B-cells by glucose results in the translocation of glucokinase towards the periphery of the cell. Furthermore, recent studies from our laboratory have shown that in rat hepatocytes glucokinase is located predominantly in the nucleus under metabolically static conditions and that metabolic conditions leading to an increase in glucose metabolism cause translocation of the enzyme from the nucleus to the cytoplasm [11,18–22]. We also suggested that the translocation of glucokinase between the nucleus and the cytoplasm plays a major role in the regulation of hepatic glucose metabolism [19–22]. It remains to be investigated whether glucokinase in D-cells is translocated between the granule and the cytoplasm in response to changes in blood glucose concentration.

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