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Immunocytochemical and ultrastructural heterogeneities of normal and glibenclamide stimulated pancreatic beta cells in the rat

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Abstract When studied morphologically in semi-thin sections in the rat in vivo, pancreatic beta cells displayed heterogeneous immunoreactivities for insulin and amylin, depending on the islet size and the intra-islet position of the beta cells. In larger islets, cortical beta cells (beta cells with contacts with all islet cell types and with the exocrine parenchyma) which are located in the periphery were more densely immunostained for insulin and amylin than medullary beta cells (beta cells with contacts only with other beta cells) which are located in the centre of the islet. Ultrastructurally, these findings were accompanied by differences in the number of secretory granules and mitochondria. Beta cells in small islets and at extra-islet sites exhibited a dense immunoreactivity. After administration of glibenclamide, immunoreactivities for insulin and amylin were diminished in a time-dependent manner, decreasing first in medullary and thereafter in cortical beta cells of larger islets. Ultrastructurally, the beta cells exhibited the typical signs of stimulation. A minority of beta cells in small islets and all beta cells in extra-islet locations remained unchanged. Thus pancreatic beta cells under basal and stimulatory conditions in vivo exhibit heterogeneity in hormone content and in ultrastructural features. These differences may represent the basis for a functional heterogeneity of the insulin secretory response of the individual beta cell both in vivo and in vitro in states of normal and impaired insulin secretion. As heterogeneity was observed only among beta cells in islets, while single beta cells surrounded by acinar cells exhibited no changes in insulin immunoreactivity, interactions between beta cells as well as between beta cells and other endocrine cells may be critical for expression of heterogeneity within the beta cell population.

Key words Rat · Pancreatic beta cells
Immunocytochemistry · Ultrastructure · Insulin

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Introduction

Pancreatic beta cells in the rat and other mammalian species exhibit a dense and uniform immunoreactivity for insulin under basal conditions [2, 30, 36]. Thus on the basis of morphological studies with paraffin sections of the pancreas beta cells have been considered to form a functionally homogeneous population in vivo. Nevertheless heterogeneities have been observed within the rat pancreatic beta cell population with respect to aldehyde fuchsin staining (granulation state) and nuclear size [15, 24]. In human pancreas beta cells have been found to exhibit heterogeneous immunoreactivity with respect to 7B2 protein [1]. On immunocytochemical analysis insulin content of human and rat beta cells proved to be inversely related to proinsulin content [26, 27]. A heterogeneous immunoreactivity of rat pancreatic beta cells for insulin was observed in vivo only after glucose stimulation [23, 36]. Under pathological conditions pancreatic beta cells in the endocrine pancreas of type I diabetic patients exhibited a heterogeneous picture. While the majority of pancreatic beta cells was destroyed a minority remained well-preserved with a dense insulin immunoreactivity [31]. Therefore the question arises whether beta cells consist of different subpopulations with variable susceptibility towards autoimmune attack [39]. Pancreatic beta cells cultured in vitro showed marked differences in the rate of insulin biosynthesis and secretion depending on the aggregation state of the isolated cells [25, 29, 33, 34]. Moreover, individual beta cells exhibited differences in their insulin secretory capacity [5, 11, 16]. Thus pancreatic beta cells studied in vitro are functionally clearly heterogeneous. In order to elucidate the reason for these discrepancies I have used semi-thin sections of plastic-embedded rat pancreas, immunostained with a highly diluted first antibody according to a standardized protocol, in order to determine the immunoreactivities of the beta cells. Under these conditions, intercellular differences in the immunoreactivity within an endocrine cell population become evident and show a stoichiometric relation to hormone content [12, 37]. Such differ-

ences have been demonstrated for example in alpha and beta cells of rabbit and cattle pancreas [10, 13]. I will show that pancreatic beta cells can be subdivided into subpopulations with differential immunoreactive staining for insulin and amylin. In addition, by the use of the consecutive semi-thin/thin section technique, the heterogeneous immunostaining was correlated with differences in the ultrastructural features such as the number of secretory granules and mitochondria. Pancreatic beta cells were examined in the pancreas obtained from control fed rats and animals after stimulation of the beta cells by a single injection of glibenclamide.

Materials and methods

Pancreatic tissue was obtained from eleven 3-month-old male Wistar rats kept under normal laboratory conditions. Four rats served as untreated controls. The other animals were treated with a single intraperitoneal injection of glibenclamide (2 mg/kg) [25, 36]. Pancreatic specimens were taken from all pancreatic regions with the exception of the duodenal region where PP-cells dominate. In the case of the glibenclamide treated animals specimens were obtained 1.5, 3, 6 or 12 h after administration.

For *light microscopy* small pancreatic specimens were quenched in melting nitrogen at −210° C, freeze-dried at −35° C for 72 h, fixed by vapour-phase *p*-formaldehyde at 60° C for 1 h, and embedded in epoxy resin (Araldite). Serial semi-thin sections were cut at 0.5 µm and mounted on microscopic slides [12].

For *electron microscopy* pancreatic specimens were fixed by immersion in a solution containing 2% *p*-formaldehyde and 2% di-glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. The tissue was post-fixed in 1% osmium tetroxide for 1 h and embedded in epoxy resin (Epon). Thin sections were cut at 50 nm on an ultramicrotome (Ultracut, Leica, Nussloch, Germany). The sections were placed on nickel grids and contrast stained with uranyl acetate and lead citrate. In addition the consecutive semi-thin/thin section technique was applied to identify heterogeneously immunostained beta cells on the ultrastructural level.

Polyclonal antisera against insulin (Insulin LAA, Novo, Bagsvaerd, Denmark or Insulin INC; Immunonuclear, USA), diluted 1:3000–1:5000, glucagon (Milab, Malmö Sweden), diluted 1:20000–1:25000, somatostatin (Dr. Etzrodt, Ulm, Germany), diluted 1:12000, and amylin (Peninsula Lab., Heidelberg, Germany), diluted 1:10000–1:20000 were used. Except for the antiserum for amylin all antisera have been used successfully in previous studies on semi-thin sections [7, 13].

Serial semi-thin sections were immunostained by the peroxidase anti-peroxidase (PAP) technique [12, 37] or by the avidin peroxidase complex (ABC) method [17]. In both methods, the resin was removed by sodium methanolate; in order to recover the antigenicities sections from osmicated tissue were etched additionally with a solution of 5% hydrogen peroxide thereafter the sections were incubated in 2% non-immune swine serum (30 min) and subsequently in the appropriately diluted first antibody (24 h at 4° C). In the PAP method, swine anti-rabbit IgG (1:20; 30 min) served as a second layer and soluble PAP complexes (1:50, 30 min) as a third layer in the protocol (both from Dakopatts, Hamburg, Germany). The ABC method stains more efficiently faint immunoreactivities for insulin and amylin in the beta cells such as regularly found after glibenclamide treatment. In the ABC method, biotinylated goat anti-rabbit IgG (1:200 for 30 min) and a streptavidin-biotin-peroxidase complex (1:1000 for 30 min) were used as second and third antibodies (both from Jackson Immuno Research, West Grove, USA). In both methods, the staining of the peroxidase was performed with 0.7 mM di-amino-benzidine (DAB) and 0.002% hydrogenperioxide in 0.05 M TRIS HCl buffer, pH 7.6. Between the various steps of the protocol, the sections were rinsed thoroughly in 0.01 M phosphate-buffered 0.5 M saline, pH 7.4.

With the exception of the antiserum for amylin all antisera have been used already in previous studies [7, 13] and their immunoreactivities have been examined for method and antibody specificities as recommended [12, 37]. In the present study the specificities of the antisera were tested by preadsorption of the antisera with their corresponding antigens and with heterologous antigens. These control experiments with all antisera confirmed the specificities of the immunocytochemical staining.

The serial semi-thin sections were immunostained by highly diluted antisera either sequentially for all pancreatic hormones or only for insulin and amylin. A total number of 1800 sections studied was composed of smaller series of 10–40 sections from different pancreatic regions of each experimental group and of larger series. One large series of 500 sections of the control group included three completely and three incompletely sectioned islets. The islets were analysed for their maximal diameters (performed by an image analysing system), their absolute and relative number of endocrine cells and especially for the distribution pattern of these endocrine cells. Determinations were carried out at intervals of 10 µm in each islet. In five series of 100 sections from the duodenal pancreas of the control and from each experimental group at least one large and one small islet were sectioned.

In order to compare beta cells in three to five islets of different size from different pancreatic regions of rats with or without glibenclamide treatment, small series of sections of all experimental groups were developed immunocytochemically in parallel. In these islets beta cells were examined for their insulin and amylin immunoreactivities with respect to pancreatic region, islet size and intra-islet position. In preliminary studies, the titres of the antisera and their suitability for quantitative immunocytochemistry were checked by running ascending dilutions of the antisera. The position of the beta cells in the islet was determined on the basis of the contacts to their neighbouring cells identified in semi-adjacent sections immunostained for glucagon and somatostatin. Cortical beta cells are defined as those with contacts to beta cells as well as to alpha and delta cells and to the exocrine parenchyma while medullary beta cells have contacts only with other beta cells. The obvious intercellular differences in the insulin immunoreactivity were determined densitometrically by the computer-assisted Interactive Image Analysis System IBAS (Zeiss-Kontron, Munich, Germany) equipped with a photomicroscope II (Zeiss, Oberkochen, Germany) and a CCD/89D video camera (Sony, Tokyo, Japan) [6, 10, 13]. The densitometric determinations were performed as single cell measurements of all beta cells of the islet sections. Thereby the intra-islet position of each beta cell can be determined. The images of the cells in a final magnification of 1:1600 were displayed on a monitor and processed by a program of densitometry (macro "densbar"). In the images of the beta cells only the areas with an immunostaining were measured by marking these areas. The densities of the insulin immunoreactivities were transferred into grey values given as arbitrary units (1=black; 225=white). When the illumination value (area without section) is constantly set to 170 (Light Control Unit LCU 2; Kontron, Munich, Germany) the mean values for the insulin immunoreactivities ranged between 80 and 140 in the control group and between 80 and 160 after glibenclamide treatment. According to these differences the beta cells were subdivided into three subtypes of densely (mean values: 80–100), moderately (mean values: 101–120) and faintly (mean values: 121–140) immunostained cells in the untreated rats; in addition, in order to demonstrate the reduced insulin immunoreactivity after glibenclamide administration in treated rats a fourth subtype representing the degranulated cells (mean values: 141–160) was used.

In order to identify the faintly and densely insulin immunostained beta cells within larger islets, five islets from different pancreatic regions were studied over a distance of 5 µm by the use of semi-thin and ultra-thin sections. In addition larger islets were investigated over a distance of 2.5 µm by sequential thin sections. In fifty cortical and medullary beta cell profiles of the control rat secretory granules and mitochondria were counted in representative areas and expressed per μm^2. These measurements were also performed in cortical and medullary beta cells of larger islets from rats 3, 6 and 12 h after glibenclamide treatment.

In all experimental groups thin sections through the same islets at different planes of sections and semi-adjacent sections through the same cells were performed. Three to five islets from different pancreatic regions were analysed in each group for various cell components, such as secretory granules and mitochondria.

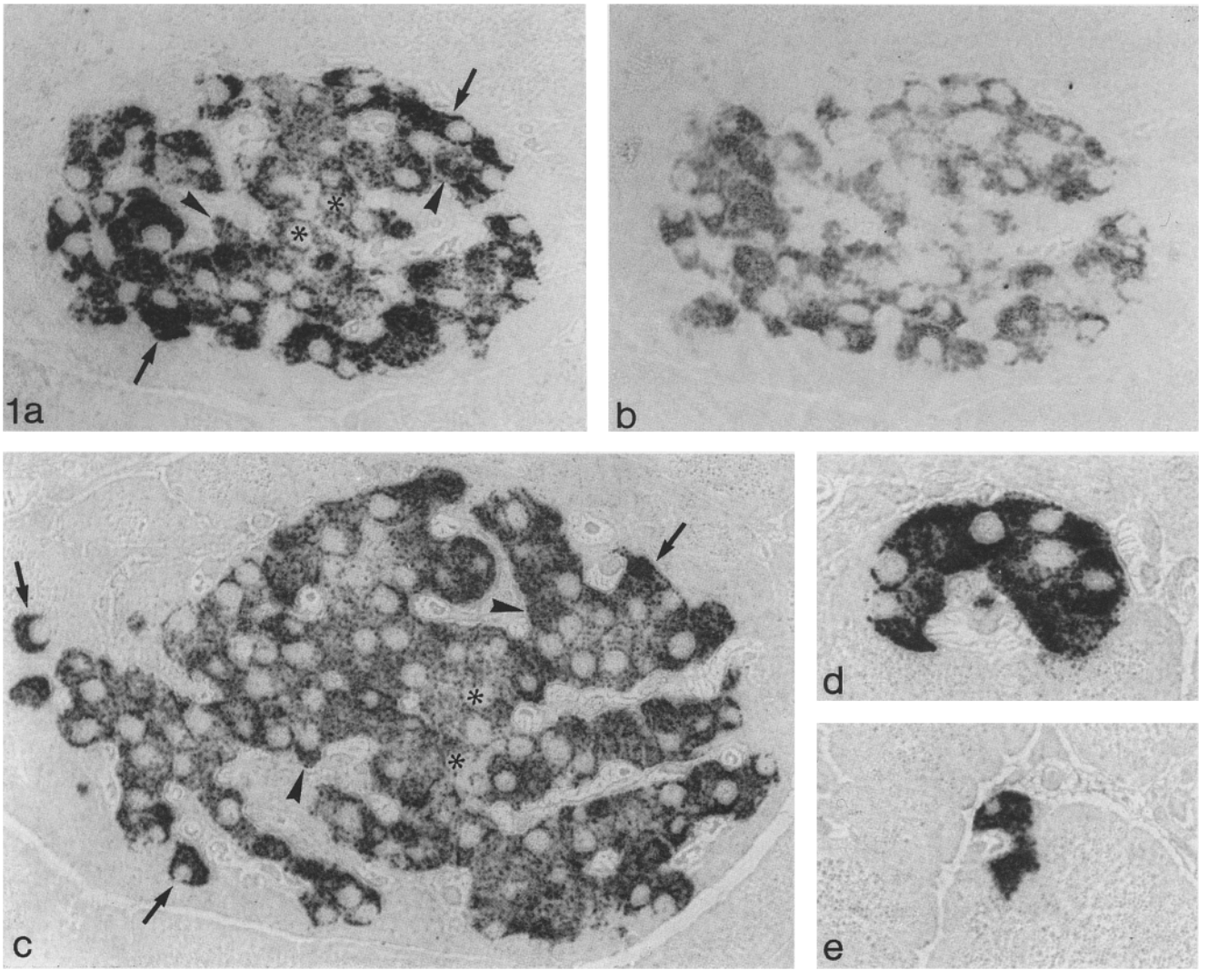
Values are presented as means±SEM. The values of the relative frequencies of the subtypes of beta cells and, in addition, the values of the secretory granules and mitochondria in Fig. 2 were tested for statistical significance with Student's *t*-test. The time-dependent reduction of both densely and faintly immunostained beta cells after glibenclamide administration in Fig. 4 was tested for significance with the analysis of variance.

Results

Beta cells in the pancreas of control rats

Morphology of the islets of Langerhans. The pancreatic islets from untreated rats differ in size and cellular composition. According to the maximal diameters, the islets are classified as small (<150 μm), medium-sized (150–250 μm), and large (>250 μm) as previously described by Bonner-Weir and Orci [3]. Irrespective of the islet size, the cellular composition varies markedly. The values for alpha cells range from 5 to 21%, for beta cells from 69 to 90% and for delta cells from 5 to 14%. The intra-islet microtopography of alpha, beta and delta cells shows a strict subdivision into a heterogeneous cortical region composed of all three cell types and a homogeneous medullary region composed of beta cells only. Thus only cortical beta cells of the islet periphery are connected to the exocrine parenchyma and possess contacts with alpha and delta cells, whereas medullary beta cells in the centre of the islet have contacts only with other beta cells.

Fig. 1a–e Insulin and amylin immunoreactivities in rat pancreatic beta cells. Semi-thin sections of a medium-sized (**a, b**), large and a small (**c, d**) islet or beta cells at an extra-islet site (**e**) of a control rat, immunostained for insulin (**a, c–e**) or amylin (**b**). Beta cells in medium-sized or large islets are heterogeneously immunostained with dense (*arrows*) moderate (*arrowheads*) or faint (*asterisks*) intensity (**c**). Beta cells in a small islet or single beta cells exhibit homogeneous dense immunoreactivity. Bright-field illumination **a–c**: ×560; **d, e**: ×720



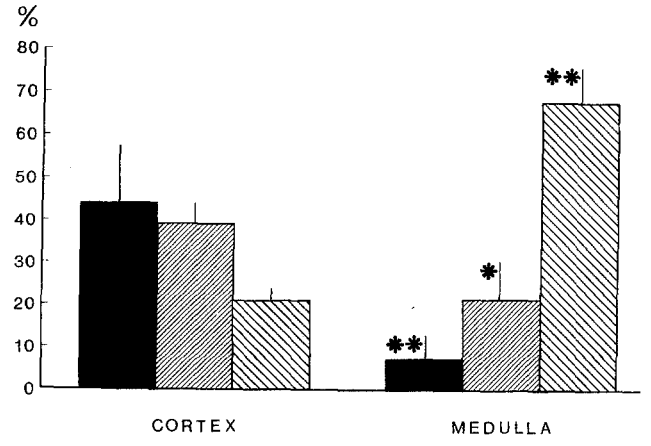


Fig. 2 Relative proportions of beta cells (mean values±SEM), classified into three groups according to the densities of their insulin immunoreactivities ("strong" (*left panel*), "moderate" (*middle panel*), "faint" (*right panel*)) and to their intra-islet position in a control rat pancreas. Insulin immunoreactivities of 225 beta cells were densitometrically determined in three sections through the same islet at 10 µm distances. In the cortex and the medulla of the islet the subtypes of beta cells are found in different frequencies. Significance: "strong" beta cells: ** $P<0.01$; "moderate" beta cells: * $P<0.05$, "faint" beta cells: ** $P<0.01$

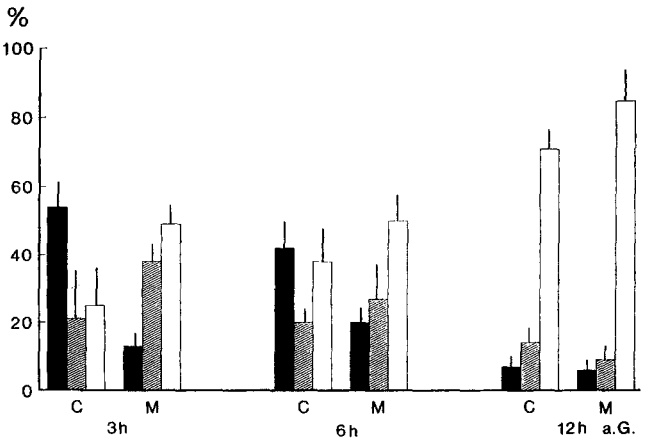
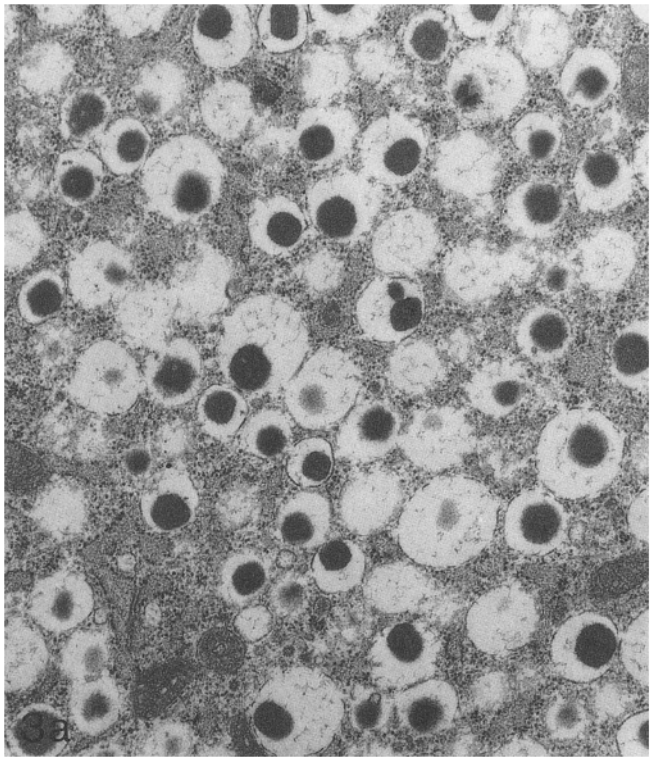
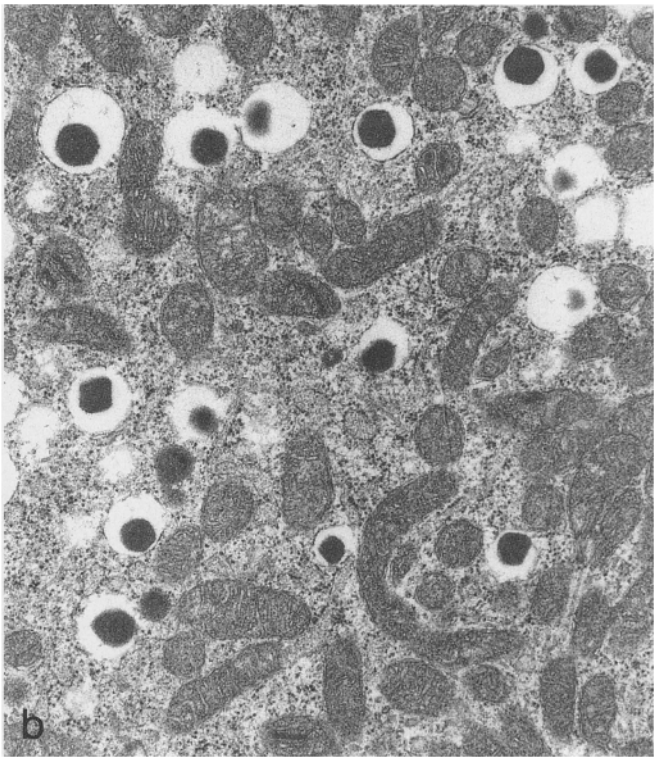


Fig. 4 Relative proportions of densely immunostained (*left panel*), faintly immunostained (*medium panel*) and degranulated beta cells (*right panel*) studied following different time intervals after glibenclamide administration (mean values±SEM). In each experimental group beta cells were densitometrically determined in three large islets with respect to the subtypes and the intra-islet position (*C*: cortex; *M:* medulla). 3 h: 187 beta cells 3 h after glibenclamide treatment; 6 h: 206 beta cells 6 h after glibenclamide treatment, and 12 h: 257 beta cells 12 h after glibenclamide treatment. The frequencies of the subtypes in beta cells after glibenclamide administration change in dependence on the time interval investigated. Compared with the controls there is a significant time-dependent reduction of both densely and faintly immunostained beta cells after glibenclamide administration ($P<0.05$; analysis of variance). Twelve hours after injection, the majority of beta cells shows a diminished insulin immunorectivity

Fig. 3a, b Ultrastructure of a cortical (**a**) and medullary (**b**) beta cell from a large islet in a control rat pancreas. Cortical and medullary beta cells exhibit notable differences in the number of secretory granules and mitochondria. **a, b**: ×20000



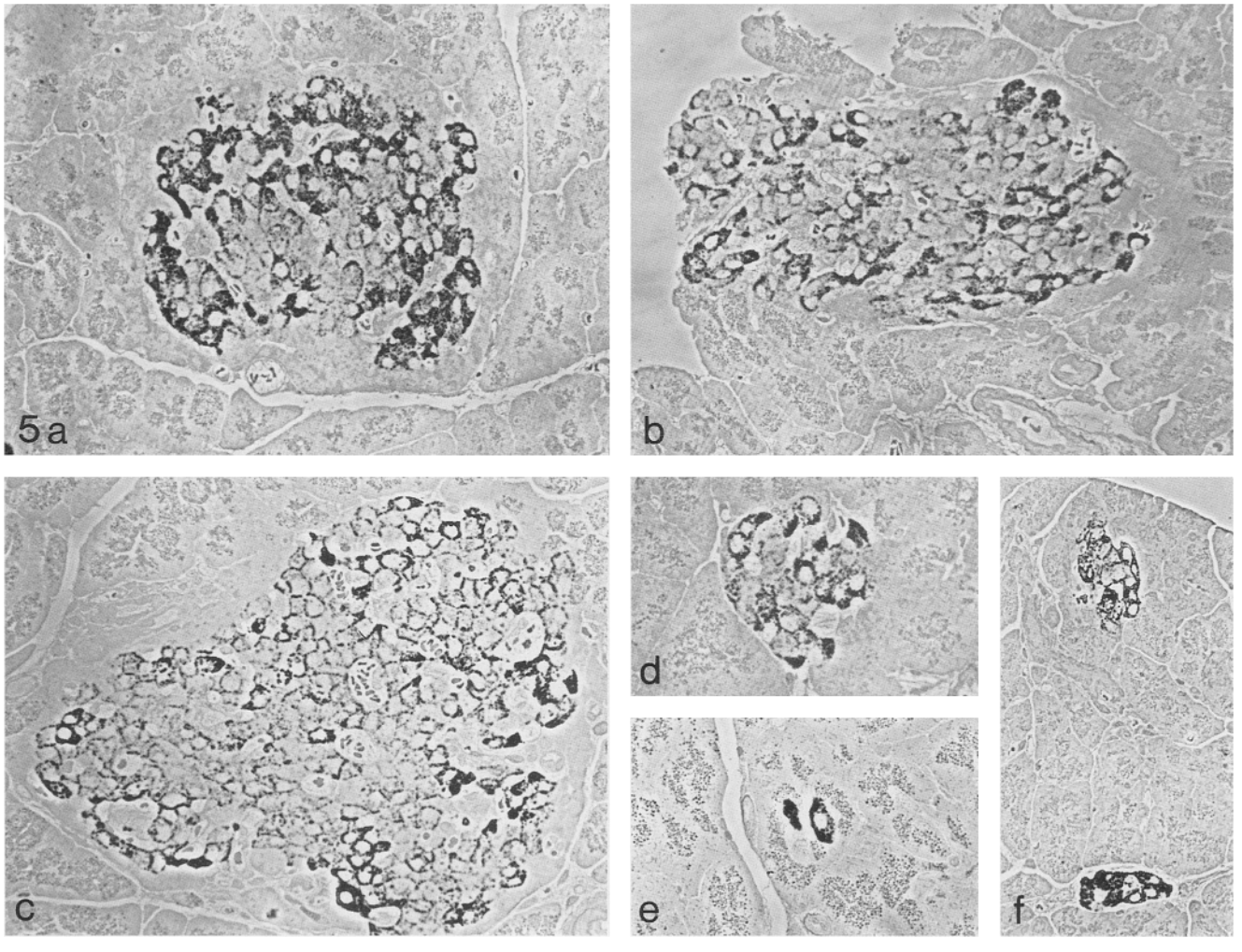


Insulin and amylin immunoreactivities. Beta cells in islets, irrespective of the pancreatic region, contain insulin and amylin immunoreactivities of varying densities (Fig. 1a–c). The heterogeneous immunostaining is observed only in beta cells of larger islets. According to the differences in the densitometrically determined insulin immunoreactivity, beta cells are subdivided into densely, moderately and faintly immunostained cells (Fig. 2). These subtypes are present both in the periphery and in the centre of larger islets with markedly different frequencies. Densely immunostained cells are localized preferentially in the islet periphery whereas medullary beta cells are immunostained predominantly faintly and only occasionally moderately or densely (Fig. 1a, c). However, a homogeneous insulin immunoreactivity is found in beta cells of small islets and at extra-islet sites. Apart from the larger islets with heterogeneously immunostained beta cells, other larger islets, rarely found in the different pancreatic regions, possess only beta cells with a uniform and dense insulin immunostaining. Beta cells in small islets (150 cells densitometrically determined) and at extra-islet positions (50 cells densitometrically determined) also display mostly dense insulin immunoreactivities (Fig. 1d, e).

In addition, beta cells contain amylin immunoreactivities with intercellular variations (Fig. 1b). The differences in the immunoreactivities for amylin parallel those for insulin. Cortical beta cells of medium-sized and large islets are mainly densely immunostained for amylin while medullary beta cells are faintly immunostained. Beta cells in small islets and at extra-islet sites exhibit dense immunoreactivity for amylin.

Ultrastructure. In order to correlate the differentially expressed insulin immunoreactivity with the ultrastructure beta cells are identified on the basis of the degree of their specific density of insulin immunoreactivity in the semi-thin section and ultrastructurally in the following thin

Fig. 5a–f Changes of insulin immunoreactivity in rat pancreatic beta cells after glibenclamide administration. Semi-thin sections of large (**a–c**) or small (**d, f**) islets from the same pancreatic region and of beta cells at an extra-islet site (**e**) 3 h (**a**), 6 h (**b, d, e**) and 12 h (**c, f**) after glibenclamide administration, immunostained for insulin. Initially immunoreactivity is diminished in medullary beta cells, thereafter in cortical beta cells in large islets and beta cells in some small islets. Beta cells at the extra-islet position are unaffected. Phase contrast **a–c**: ×300, **d, e**: ×360, **f**: ×225



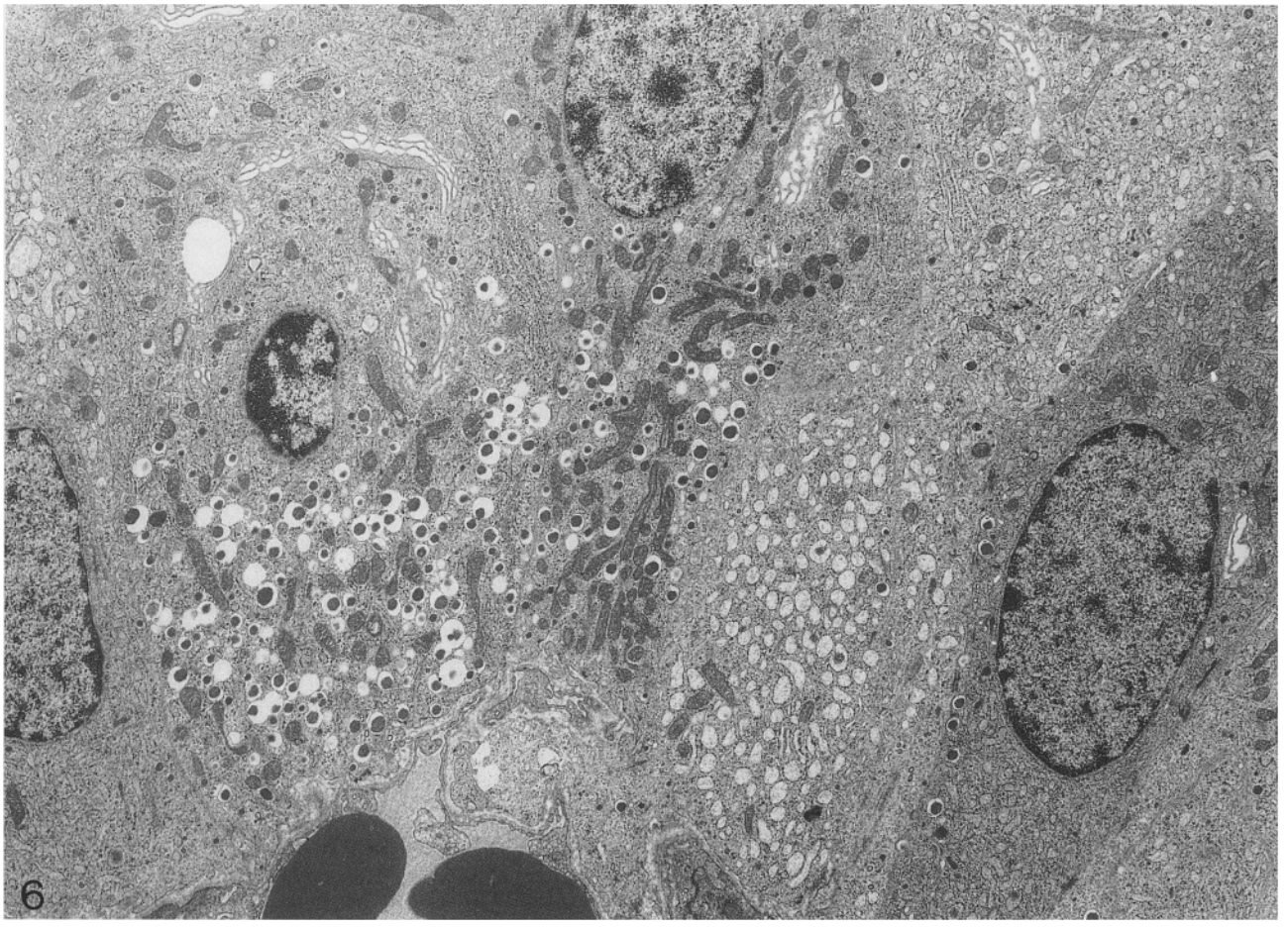
section. Beta cells exhibit no differences in size or development of the rough endoplasmic reticulum or the Golgi complex. But the number of secretory granules and of mitochondria varies markedly among cortical and medullary beta cells of larger islets (Fig. 3a, b). In cortical beta cells the number of secretory granules and mitochondria is 69±18 and 12±4, respectively. In comparison, in medullary beta cells the number of secretory granules is significantly lower (31±11; $P<0.05$), while the number of mitochondria is significantly higher (25±14; $P<0.05$). Some of the medullary beta cells are even completely degranulated. In addition a few beta cells are poorly granulated in the cortex and well granulated in the medulla.

Beta cells in the pancreas of glibenclamide treated rats

Morphology of the islets of Langerhans. Islet size and cellular composition are not affected when the compound is administered to the rats at various times before fixation of the pancreas. The changes of the heterogeneities after glibenclamide stimulation are similar in pancreatic beta cells in islets irrrespective of the pancreatic region, while no changes are detected in non-beta cells.

Insulin and amylin immunoreactivities. Following treatment with glibenclamide, the insulin and amylin immunoreactivities are diminished in beta cells. The time course of the effects on insulin immunoreactivity as shown in Fig. 4 is determined densitometrically in beta cells of larger islets. Three hours after glibenclamide administration medullary beta cells of medium-sized and large islets are the first subpopulation to exhibit fainter immunoreactivities for insulin and amylin (Fig. 5a). Cortical beta cells remain densely immunostained for insulin and amylin. Six hours after glibenclamide administration, some of the cortical beta cells in larger islets also show decreased insulin and amylin immunoreactivity (Fig. 5b). Some beta cells of small islets are fainter than control cells (Fig. 5d). Twelve hours after glibenclamide administration, the majority of beta cells in large islets displays only weak immunoreactivities for insulin and amylin (Fig. 5c). For both constituents the immunoreactivity is mostly restricted to the plasmalemmal region. A minority of the beta cells, irrespective of the islet region, preserves a strong insulin immunoreactivity. In addition there are a few islets of small and larger size showing no alterations

Fig. 6 Ultrastructure of beta cells in the medulla of a large islet of the rat pancreas 6 h after glibenclamide treatment. The development of the amount of the rough endoplasmic reticulum, the Golgi-complex, the mitochondria and the secretory granulation varied among the stimulated beta cells. ×7200



(Fig. 5f). Beta cells at extra-islet sites also remain unchanged at all times after glibenclamide administration (Fig. 5e).

Ultrastructure. In accordance with the immunocytochemical findings, ultrastructural changes are heterogeneous in beta cells of islets with different size and develop after glibenclamide administration in a time-dependent manner. Initially only medullary beta cells of larger islets show distinct alterations in the cytological features to be followed thereafter by similar changes in the cortical beta cells of the same islets and in the beta cells of small islets. Interestingly, the glibenclamide stimulated beta cells exhibit individual changes in the number and size of the different cell organelles (Fig. 6), which are less pronounced 3 h than 6 or 12 h after administration. The number of secretory granules varies markedly, ranging from a slight decrease to a complete degranulation. In medullary beta cells the number of secretory granules is significantly reduced after three hours (14±10; $P<0.05$) and in cortical beta cells after 6 h (38±14; $P<0.05$). Twelve hours after glibenclamide administration the majority of medullary and cortical beta cells is degranulated (13±10, $P<0.01$). In addition to a variable increase of the Golgi-complex beta cells possess a rough endoplasmic reticulum with small or dilated cisternae, again with considerable intercellular variation. An increasing number of mitochondria makes this organelle the main contituent of some activated beta cells. In contrast, beta cells at extra-islet sites are unstimulated and remain well granulated.

Discussion

Heterogeneity among beta cells is present both under basal and stimulatory conditions in vivo as shown by the experiments with glibenclamide treatment of the rat. This confirms the assumption that heterogeneity of pancreatic beta cells is not purely an in vitro phenomenon [30]. In previous studies, using paraffin sections of the pancreas, a high and uniform density of insulin immunoreactivity in the pancreatic beta cells was observed under basal conditions [8, 32]. After administration of glucose or glibenclamide to the rat, heterogeneity was detected among beta cells [36]. Therefore, it has been assumed that beta cells might exhibit functional heterogeneity in vivo only after stimulation [2, 30]. In contrast to paraffin sections, semi-thin sections detect only one layer of secretory granules and layers of secretory granules will not obscure intercellular differences in immunoreactivity [12, 13], and thus it was possible to demonstrate heterogeneous immunoreactivities for insulin and amylin in beta cells under basal conditions in this study. Intercellular differences in insulin immunoreactivity have previously been observed in beta cells of men and rabbits [13, 19]. From studies using serial ultra-thin sections it is known that secretory granules are distributed equally within the cell, so that there is apparently no polarity of secretory granule distribution in non-stimulated beta cells [42]. Heterogeneous insulin and amylin immunoreactivities as observed in the present study in both cortical and medullary beta cells can therefore be correlated with ultrastructural variations in the number of secretory granules and mitochondria. Changes in the number of these cell organelles indicate differences in the functional activity of the beta cells. Such differences have been observed previously, also in vitro, under different functional states [35, 42]. Morphological heterogeneity clearly implies that the beta cells in the islets belong to functionally heterogeneous subpopulations.

To demonstrate the existence of functional heterogeneity among pancreatic beta cells in vivo under conditions of stimulation beta cells were stimulated with the sulphonylurea drug glibenclamide, which is a potent stimulator of insulin secretion. Glibenclamide treatment is known to cause a marked decrease in aldehyde fuchsin staining and in the number of secretory granules [21, 28, 38]. Bonner-Weir [2] has reported polarity of secretory granules in glibenclamide stimulated beta cells. In the present study I detected a polarity of the secretory granule distribution in certain areas of the beta cells. Ultrastructurally, fainter insulin immunoreactivity in response to glibenclamide treatment is paralleled by an increase in the size or number of cell organelles responsible for the synthesis of insulin [9, 40, 41]. In a previous study on paraffin sections of the pancreas from glibenclamide treated rats heterogeneously diminished insulin immunofluorescence was found in cortical and medullary beta cells of splenic islets [36]. The present study confirmed these observations, showing that the glibenclamide induced changes in beta cells are time-dependent and vary with respect to islet size and intra-islet localization of the beta cell. The medullary and the cortical beta cells in larger and some of the small islets represent cell subpopulations with secretory activity. Subpopulations of beta cells with different sensitivity for glucose have been described by other authors [23, 36]. The pancreatic beta cells with unchanged immunoreactivity represent the non-sensitive subpopulation or cells in a resting state. Such non-sensitive beta cells have also been observed after short-term glucose stimulation [4]. The existence of functionally heterogeneous subpopulations may be important also for the initial morphological changes during the onset of diabetes. The secretory active and the resting beta cells may differ in their susceptibility towards cytotoxic damage. Using Chinese hamsters as a model of type II diabetes the subpopulation of surviving beta cells was characterized by a dense immunoreactivity for insulin and a moderate immunoreactivity for the GLUT2 glucose transporter [20].

Functionally heterogeneous beta cell subpopulations are also known to exist under in vitro conditions [2, 29]. Beta cells show differences in the rate of insulin secretion, depending on the aggregation state. Beta cells in clusters secrete more insulin per cell than single beta cells [29, 33]. Single cells obtained from different islets also vary in their rate of insulin release [5, 11, 16]. Differences in insulin secretory responsiveness have been

shown to correlate with differences in the redox state [22] and in the rate of glucose phosphorylation [14]. Thus differences in the metabolic activity of the beta cells have been considered to be responsible for functional heterogeneity. Most recent studies show that variations in glucose phosphorylation due to differential glucokinase gene expression rather than in glucose transport via the GLUT2 glucose transporter might be the metabolic basis for heterogeneity of pancreatic beta cells [14, 18]. Thus the reason for the observed heterogeneity might reside in the initial phosphorylation step of glucose rather than in glucose transport. The microenvironment of the pancreatic beta cell within the islets is determined by a multitude of internal and external factors which regulate pancreatic islet function via endocrine, paracrine or neural mechanisms. Though it is not possible to delineate a single factor responsible for beta cell heterogeneity, certain conclusions can be drawn on the basis of the intrapancreatic and intra-islet topography of the beta cells. A heterogeneity of pancreatic beta cells was observed in medium-sized and large islets in all regions of the pancreas. Surprisingly, however, all beta cells in small islets and single beta cells at extra-islet positions presented a consistently homogeneous and dense insulin immunoreactivity which was not affected by administration of glibenclamide to the animal. Because active beta cells were registered consistently in the larger islets from different pancreatic regions both heterologous cell contacts of beta cells in the cortical region and homologous cell contacts in the medullary region may affect the pattern of heterogeneity. Contacts of the beta cells to the intra-islet capillary system were the same in cortical and medullary regions of the islet as observed in a 3-D-reconstruction of a completely sectioned islet (unpublished observation).

In conclusion, heterogeneity of pancreatic beta cells in vivo with respect to the immunoreactivities for insulin and amylin as well as to the number of secretory granules and mitochondria was demonstrated in a morphological analysis of semithin and consecutive semi-thin and ultra-thin sections. The present study demonstrates that beta cells in the pancreatic islet in vivo exhibit differences in their immunoreactivities for insulin and amylin as well as in the number of secretory granules and mitochondria. This heterogeneity may be regarded as the morphological correlate of intercellular variations in glucose responsiveness of the beta cells under basal and stimulatory conditions, and for differences in susceptibility to cytotoxic damage.

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