

## THE CAPABILITY FOR REGULATION OF INSULIN SECRETION BY SOMATOSTATIN IN PURIFIED PANCREATIC ISLET B CELLS DURING AGING\*

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### SUMMARY

Pancreatic islet B cells from Sprague–Dawley and Fisher 344 rats aged 3–27 months were separated from A and D cells by centrifugation over a linear percoll density gradient, and incubated *in vitro* with various concentrations of glucose and somatostatin. Elevation of glucose concentration in the incubation medium from 2.6 to 16.7 mM provokes an insulin secretory response that is independent of rat donor age. Inhibition of the insulin secretory response by somatostatin is independent of rat donor age beyond 12 months. These data indicate that the impaired regulation of insulin secretion during aging observed previously *in vivo* and *in vitro* in intact islets may not be intrinsic to the B cells, but instead reflect changes in islet paracrine regulatory mechanisms that relate to the quality and/or quantity of endogenous somatostatin and/or glucagon.

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*Key words:* Insulin; Somatostatin; Glucose; B cell; Paracrine; Rats

### INTRODUCTION

Impairments in glucose-stimulated insulin response that are detected in portal vein blood of aging rats [1] also are expressed *in vitro* when pancreatic islets of Langerhans

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are isolated from rat donors of increasing age [2–4]. Subsequent mechanistic pursuits focussed primarily on molecular events which occur in B cells of intact islets, such as insulin biosynthesis and processing, glucose metabolism, and adenylate cyclase activity [2,5–9]. However, treatment of isolated islets from aged rats with somatostatin antibodies restores a substantial portion of the glucose-stimulated insulin response [10]. Furthermore, somatostatin secretion by isolated islets increases with rat donor age [10]. This suggests that certain age-associated events in B cells of intact islets may be secondary to changes in the regulation of glucose-stimulated secretion of insulin by endogenous somatostatin, as well as by other paracrine factors. The present study was undertaken to assess the capability of purified islet B cells from rats of different ages for glucose-stimulated secretion of insulin in the presence and absence of added somatostatin.

## MATERIALS AND METHODS

### *Isolation of pancreatic islets and B cells*

Male Sprague–Dawley and Fisher 344 rats were obtained from the Charles River Breeding Laboratories in Wilmington, MA at 2–4, 9–12, and 24–27 months of age. Conditions of maintenance at commercial and university animal facilities, as well as relevant pathology and mortality data, were described previously [11,12]. Removal of pancreas immediately following decapitation and isolation of pancreatic islets of Langerhans by collagenase digestion were accomplished by modifications [13] of the methods of Lacy and Kostianovsky [14].

A mixed cell suspension containing A, B, and D cells was isolated from intact islets by modification of the method of Pipeleers and Pipeleers-Mirichal [15]. B cells were separated from A and D cells as follows. The mixed islet cell suspension was loaded at a concentration of  $1.0\text{--}1.5 \times 10^3$  cells/ml onto a linear gradient of percoll and centrifuged for 5 min at 2000 g. Various density fractions were separated according to the distribution of density marker beads. Cells were concentrated by centrifugation at 150 g for 10 min. Recovery of cells was determined by hemocytometric analyses of numbers of cells in the loaded mixed islet cell suspension and in each density fraction immediately prior and subsequent to density gradient centrifugation. Viability was determined as the percentage of cells that do not stain with Trypan Blue dye in any sample.

Identification of A, B, and D cells was determined immunohistochemically, as well as by chemical extraction of glucagon, insulin and somatostatin. Formalin-fixed cells taken from the first density fraction of the percoll-gradient were stained by immunoperoxidase using the avidin-biotin complex method (ABC) with an unlabeled primary antibody, biotinylated secondary antibody, and a preformed avidin-biotin-horseradish peroxidase complex (Vector Labs., Burlingame, CA). Cells were treated with diaminobenzidine and counterstained with hematoxylin [16]. Following density gradient centrifugation, each separated density fraction was extracted for 20 h in absolute ethanol/water/concentrated HCl in volume proportions of 150:47:3, and assessed for content

of glucagon, insulin, and somatostatin. The concentration of insulin was determined by specific solid phase radioimmunoassay [2]. Rat insulin standards were obtained from NOVO (Copenhagen), and [ $^{125}$ I]insulin from New England Nuclear. The inter-assay variation was 6% and intra-assay variation, 3%.

Radioimmunoassay of somatostatin was performed according to the procedure described by Gerich *et al.* [17]. Synthetic somatostatin standards were purchased from Beckman and [ $^{125}$ I]somatostatin from New England Nuclear. The antisera was a generous gift from Dr. J. Gerich, the Mayo Clinic. This antibody responds with 50% binding at 1:12 000 to 1:50 000 final dilution. Separation of bound and free somatostatin was accomplished by addition of 500  $\mu$ l dextran-coated charcoal. The intra-assay variability was about 10% and the interassay variability was about 16%.

The radioimmunoassay for glucagon was performed by the University of Michigan Diabetes Research and Training Center. Beef-pork glucagon was used as  $^{125}$ I-labelled trace (New England Nuclear) and standard. Separation of the bound and free fractions was accomplished by precipitation with a second antibody (Ann Arbor). The interassay variability was 10.2% and intraassay variability was 7.5% [18].

#### *Insulin secretion studies*

Percoll density gradient fraction 1 contained the greatest amount of B cells, and was utilized for all studies of insulin secretion. Cell suspensions containing 4000 B cells in 0.1 ml were preincubated for 30 min with 1.0 ml Krebs buffer containing 2.67 mM glucose at 37°C and 95/5% O<sub>2</sub>/CO<sub>2</sub>. Cells were pelleted by centrifugation at 150 g at 4°C for 10 min. Cell-free aliquots of the solution were assayed for zero-time content of insulin. The pelleted cells were washed twice with the same solution, and then were suspended in solutions containing varying amounts of glucose and somatostatin. Following a 1-h incubation under conditions identical to those of the preincubation, cells were pelleted by centrifugation, and the cell-free solution was assayed for insulin content, as was the cell pellet following extraction with acid ethanol.

#### *Statistical analysis*

The different treatment groups and age groups were compared by the BMDP mixed effects three-way analysis of variance (BMDP3V). *P* values were adjusted using the Bonferroni Multiple Comparisons Procedure [19].

### EXPERIMENTAL RESULTS

The distribution of extractable insulin, glucagon, and somatostatin among seven percoll density gradient fractions is illustrated in Fig. 1 for 2–3-month-old Sprague–Dawley rats. Fraction 1 contains slightly more than 60% of the total insulin and less than 10% of the total glucagon and somatostatin recovered from the entire gradient. Insulin content in fraction 2 decreases to less than 25% of total recovered insulin, and the remaining 10–15% of the extractable insulin is distributed among fractions 3–7.

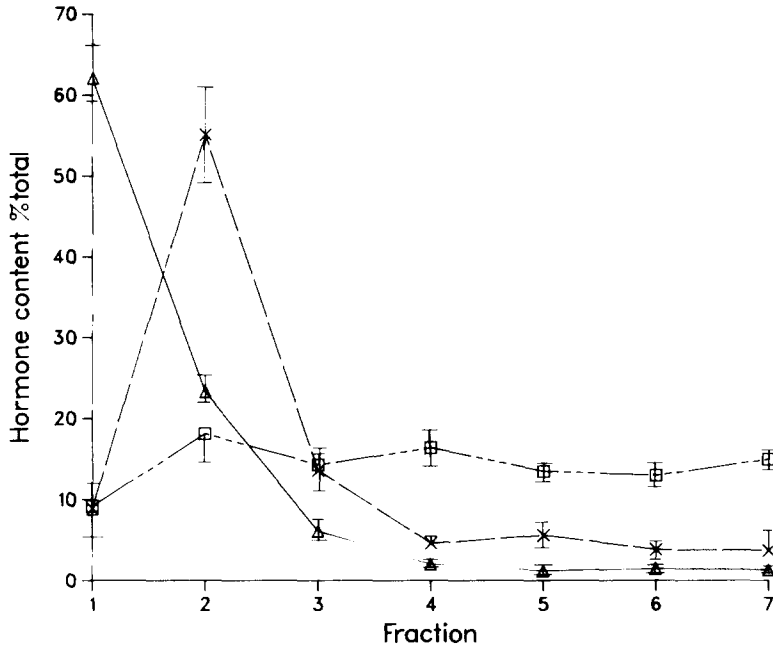


Fig. 1. Extractable hormone distribution in density gradient fractions. Mixed islet cell suspensions were prepared from pancreases of 2-month-old Sprague-Dawley rats, and separated by Percoll density gradient centrifugation into seven fractions. The cells from each density gradient fraction were extracted in acid-ethanol and assayed for content of insulin, glucagon, and somatostatin. Data are expressed as the mean percentage  $\pm$  S.E.M. of each respective total hormone extracted from the entire Percoll gradient for seven different cell preparations.  $\Delta$ , insulin;  $\times$ , glucagon;  $\square$ , somatostatin.

Extractable glucagon is found primarily in fraction 2, and extractable somatostatin apparently is distributed approximately equally among the seven gradient fractions. Immunohistochemical analysis of the cells in fraction 1 revealed the following distribution: B (85%); A (13%), and D (2%). This cell distribution did not change between 2 and 27 months of age. Although data are not presented, the patterns of density gradient fractionation are identical at 2, 12, 18 and 27 months of age in Sprague-Dawley and Fisher rats.

The effect of glucose and somatostatin on secretion of insulin by partially purified islet B cells from rat donors of different ages is indicated in Table I. The amount of insulin secretion per cell preparation during a 1-h period varies as much as 10-fold from one preparation to another in response to 2.6 mM glucose. However, when effects of higher glucose concentration and somatostatin on each cell preparation are normalized with respect to its insulin secretion in response to 2.6 mM glucose, significant trends are apparent. Elevation of the glucose concentration in the incubation medium to 16.7 mM increases the amount of insulin secretion by approximately 40% by B cells from donor rats aged 2–27 months. Somatostatin inhibits the insulin response to 16.7 mM glucose

TABLE I

## INHIBITION OF GLUCOSE-STIMULATED SECRETION OF INSULIN BY SOMATOSTATIN

<i>Somatostatin concentration (ng/ml)</i>	<i>Age (months)</i>	<i>Insulin secretion (percent response)</i>
0	2-3	145 ± 7.2 (a)
0	12-27	134 ± 7.5 (b)
1	2-3	131 ± 9.0 (c)
1	12-27	116 ± 4.0 (d)
5	2-3	102 ± 4.0 (e)
5	12-27	112 ± 4.0 (f)
10	2-3	91 ± 7.5 (g)
10	12-27	94 ± 7.0 (h)

Insulin secretion is expressed as the normalized 60-min response to 16.7 mM glucose relative to that of 2.6 mM glucose by the identical cell preparation. Mean values ± S.E.M. were calculated for six separate cell preparations at ages 2-3, 12-15, 18-21, and 24-27 months. Values for the three oldest groups were combined because they were identical. *P* values refer to the lower case letters following each insulin response:

- b vs. d, *P* < 0.01.
- b vs. f, *P* < 0.01.
- b vs. h, *P* < 0.01.
- a vs. c, not significant.
- a vs. e, *P* < 0.001.
- a vs. g, *P* < 0.001.

similarly by B cells from donor rats of different ages, although there is a small, statistically significant increase in sensitivity to the lowest utilized concentration of somatostatin by cells from older rats. For all of the data presented in Table I, values were identical using cells from Sprague-Dawley and Fisher 344 rat donors at 12, 18, and 27 months of age. Therefore, these values were combined and expressed as mean ± S.E.M. for old rats.

## DISCUSSION AND CONCLUSIONS

*Partial purification of B cells*

Percoll density gradient centrifugation provides a convenient, rapid and reproducible method for separation of B cells with respect to A and D cells. The density of B cells (1.065 g/ml) is less than that of A (1.068 g/ml) and D (1.070 g/ml) cells. Therefore, B cells remain at the top of the gradient.

Separation of B cells on the gradient requires approximately 5 min, as compared with 35 min on the elutriator [15]. Percentage recovery (75%) and viability (90%) are similar utilizing either method. Under the light microscope the B cells appear undamaged and primarily in single particle form.

### *Insulin secretion*

When the concentration of glucose in the incubation medium is elevated from 2.7 to 16.7 mM, partially purified B cells from rats aged 2–27 months increase insulin secretion by approximately 40%. This magnitude of insulin response is considerably smaller than that observed previously for intact islets by this laboratory [2,10], as well as by others [3]. However, such responsiveness is in agreement with results of others who examined dispersed islet cells [20–24]. These data lend additional support to the concept that paracrine interrelationships which characterize intact islets are important to glucose-stimulated secretion of insulin.

Inhibition of insulin secretion by somatostatin provides credibility for the physiological integrity of the partially purified B cell preparation that is described in the present article. Apparently, the inability of earlier studies to demonstrate the inhibitory effect of somatostatin on isolated B cells [25–27] is overcome when islet digestion is performed with trypsin-free collagenase.

### *Impact of aging*

Isolated B cells from donor rats aged 3–27 months show no difference in magnitude of insulin secretory response to a glucose challenge. Furthermore, somatostatin inhibits insulin secretion similarly by isolated B cells from rats of different ages. The small increase in sensitivity to inhibition at the lowest utilized concentration of somatostatin is expressed between 2 and 12 months of age, and probably does not relate causally to differences that were observed previously at much later points in the lifespan. Therefore, differences in glucose-stimulated secretion of insulin during aging probably are secondary to alterations in islet paracrine control mechanisms which address the quality and/or quantity of endogenous glucagon and/or somatostatin that is available to B cells within intact islets.

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