Altered Cellular Heterogeneity as a Possible Mechanism for the Maintenance of Organ Function in Senescent Animals

Rodney C. Ruhe,¹ Donald L. Curry,² and Roger B. McDonald¹

¹Department of Nutrition, University of California, Davis.
²College of Graduate Studies, Middle Tennessee State University, Murfreesboro.

We tested the hypothesis that an alteration in the functional heterogeneity of cell populations (i.e., changes occurring in sensitivity and responsiveness to external stimuli among individual cells) may be a mechanism by which some organs are able to resist age-related decrements in function. To this end, changes in cytoplasmic free calcium concentration ([Ca²⁺]), following glucose stimulation of individual pancreatic β cells isolated from male F344 rats of ages 6, 12, and 26 mo were used as a model for evaluating responsiveness and sensitivity. Changes in [Ca²⁺] of individual β cells were monitored using fura-2 microspectrofluorimetry. No differences were observed in [Ca²⁺], or in insulin secretion per β cell among the age groups at any of the glucose concentrations. However, the percentage of β cells that were responsive to a stimulatory glucose concentration (>5.5 mM) was significantly greater in islets from the 26-mo-old rats (76%) as compared to the 6- and 12-mo-old animals (63% and 65%, respectively). Of the responsive β cells, a significantly greater percentage of those from the 26-mo-old rats (72%) responded at the lowest stimulatory glucose concentration (7.5 mM) as compared to the 6- and 12-mo-old animals (58% and 60%, respectively). These data suggest that the maintenance of organ function in older rats at a level comparable to that of younger animals may be accomplished, in part, by an increase in the percentage of cells that are responsive to stimuli and/or by an increase in the sensitivity of the responsive cells.

THEORIES concerning the process of aging, whether at the physiological, cellular, or molecular level, tend to predict a gradual, inexorable, and uniform decline in tissue function (Warner et al., 1987). Although some organs and physiological systems do exhibit well-defined decrements in function in the senescent mammal, the performance of others remains relatively unchanged throughout the life span of the animal. For example, neural structure and many aspects of brain metabolism have been shown not to diminish significantly with age (Cotman, 1990). We have demonstrated that nonshivering thermogenesis of brown adipose tissue is reduced significantly in aged animals that exhibit no loss in insulin secretory capacity (McDonald et al., 1988; Gabaldon et al., 1995). These results pose the following question: What mechanism may allow a particular cell or cell population to resist the deleterious effects of aging, while within the same senescent environment, the activity of other cell populations is diminished? In this investigation we hypothesize that an alteration in the functional heterogeneity of cells within an organ — i.e., changes occurring in sensitivity and responsiveness to extrinsic stimuli among individual cells — may be a mechanism by which some organs are able to resist age-related decrements in function.

In the present investigation, the pancreatic β cell was used as a model for cell populations that are able to maintain appropriate function throughout the life span of the organism. The β cell is particularly useful for evaluating alterations in functional heterogeneity as a means of resisting the deleterious effects of aging for two reasons. First, several investigations have shown that in senescent animals exhibiting diminished function of various organs, insulin secretion is not altered significantly (Burch et al., 1984; Leiter et al., 1988; Starnes et al., 1991; Ruhe et al., 1992). Second, recent studies in younger animals have indicated that the pancreatic β-cell population is heterogeneous with respect to glucose responsiveness. That is, Pipeleers (1992) and Wang et al. (1993) have reported that approximately 30% of the β-cell population did not respond to glucose in terms of insulin secretion and an increase in cytoplasmic free calcium concentration, even though these cells were morphologically similar to the 70% of cells that did respond to glucose. The sensitivity of these glucose responsive cells — i.e., the concentration of glucose at which they respond — has also been shown to differ among individual cells. In light of these observations, we suggest that the maintenance of insulin secretion in the aging animal may reflect a change in the relative proportion of glucose-responsive to glucose-nonresponsive β cells and/or a change in the sensitivity of those cells that are responsive to glucose.

To evaluate changes in the functional heterogeneity of the pancreatic β cell, we measured cytoplasmic free calcium concentration ([Ca²⁺]) following glucose stimulation. The responsiveness of the β cell to glucose is linked directly to an increase in [Ca²⁺]. (Wollheim and Sharp, 1981; Prentki et al., 1987). Previous studies have demonstrated that other important steps in the signal transduction pathway of insulin secretion, particularly glucose oxidation and the closure of ATP-sensitive potassium channels, are unaffected by age (Burch et al., 1984; Leiter et al., 1988; Ruhe et al., 1992, 1993). However, the effects of age on calcium regulation by the β cells have not been examined as thoroughly. A report by Ammon et al. (1987) indicated that the glucose...
stimulated influx of calcium was diminished in islets from older rats as compared to islets from younger animals. It is thus possible that an age-related alteration in β-cell calcium regulation may be associated with the degree to which the aging cell is sensitive to glucose.

The aim of the present investigation was to evaluate a possible mechanism to explain the disparity among various tissues with respect to age-related changes in functional integrity. In this regard, we have tested the hypothesis that the maintenance of pancreatic β-cell function during senescence results from alterations in the functional heterogeneity of the β-cell population, as determined by monitoring changes in \([Ca^{2+}]_i\), of individual β cells during glucose stimulation. We have also examined the relationship between \([Ca^{2+}]_i\), and glucose responsiveness/sensitivity of these cells, and the effects of aging on this relationship.

**MATERIALS AND METHODS**

**Animals and animal care.** — Male inbred F344 rats of ages 5, 11, and 25 mo were obtained from the National Institute on Aging animal colony maintained by Harlan Sprague Dawley Laboratory (Indianapolis, IN). Upon arrival, the animals were placed in a laminar flow unit (Duos-Flo; Laboratory Products, Maywood, NJ) that circulates air through filters at a constant rate providing clean air. All rats were housed individually in wire-bottomed hanging cages (20 x 25 x 18 cm) and maintained on a 12:12 h light/dark cycle (lights on at 0600 h, lights off at 1800 h) at a room temperature of 25–26 °C. Rats were fed NIH-31 laboratory chow (Teklad Research Diets, Indianapolis, IN) and given distilled water ad libitum. Rats were maintained in our colony for approximately 1 mo before experimental procedures. That is, rats were 6 (n = 5), 12 (n = 5), and 26 (n = 7) mo of age at the start of the experiment. Only animals showing no signs of overt disease were used in these experiments.

**Islet isolation and preparation of β cells.** — Pancreatic islets of Langerhans were isolated by the collagenase digestion technique of Lacy and Kostianovsky (1967) as modified by Ruhe et al. (1992). Briefly, the pancreata of pentobarbital sodium-anesthetized rats (65 mg/kg i.p.) were infused via the common bile duct with 13 ml cold Krebs Ringer bicarbonate (KRB) medium containing 0.75 mg/ml collagenase (Sigma, St. Louis, MO; Type XI). The distended pancreas was dissected free, placed into 7 ml of the collagenase solution, and incubated in a 37 °C water bath. The collagenase-digested tissue was placed into ice-cold KRB medium and dispersed by gentle aspiration with a 5-ml plastic pipette. The tissue was washed three times in ice-cold KRB medium, centrifuged at 200 x g for 1 min, and filtered through a wire screen (opening size, 850 μm). Islets in the range of 90–110 μm diameter were picked by hand using a dissecting microscope equipped with an ocular micrometer. The islets were maintained at 37 °C in tissue culture medium [RPMI 1640 medium (JRH Bioscience, Woodland, CA)] supplemented with 5.5 mM glucose, 2 mM L-glutamine, 10% (vol/vol) heat-inactivated fetal bovine serum (JRH Bioscience), 100 units/ml penicillin, and 100 μg/ml streptomycin (Sigma) under an atmosphere of 95% air/5% CO2. After 1–7 days in culture, the islets were dissociated into individual islet cells by incubation in a Ca2+-free KRB medium containing trypsin (Boehringer Mannheim; 25 μg/ml) and 1 mM EGTA for 8 min at 37 °C. Viability of dispersed islet cells was 90–95% as determined by trypan blue exclusion. A pilot study demonstrated that insulin secretion and changes in \([Ca^{2+}]_i\), in response to glucose stimulation were maintained through at least the tenth day of islet culture.

**Measurement of cytoplasmic free calcium.** — The \([Ca^{2+}]_i\), of individual β cells was measured fluorometrically using the Ca2+-sensitive fluorescent dye fura-2 (Grynkiewicz et al., 1985). Dispersed islet cells were placed into KRB medium containing 5.5 mM glucose and 1 μM fura-2 acetoxyxymethylster (Molecular Probes, Eugene, OR) at 25 °C for 15 min. The fura-2-loaded cells were washed and resuspended in the KRB medium, and 100 μl of the suspension was placed into a tubular plastic chamber (500 μl total volume) affixed to a glass coverslip. The chamber was placed on the stage of an inverted Nikon microscope and maintained at 37 °C. After a 10-min preincubation, during which time the cells were allowed to adhere to the coverslip, 100 μl of KRB containing an amount of glucose sufficient to yield a final concentration of 7.5 mM was added to the chamber. The cells were incubated for 20 min, followed by two consecutive 20-min incubation periods in which the medium glucose concentrations were increased to 11.1 and 16.7 mM, respectively. The \([Ca^{2+}]_i\), of individual β cells was measured with dual excitation wavelength (340 and 380 nm; emission monitored at 500–530 nm) microfluorimetry performed with a Photon Technology International Inc. (South Brunswick, NJ), DeltaScan instrument. Images of the cells were recorded at 2-min intervals throughout the incubation periods using a CCD camera (Hamamatsu, Middlesex, NJ). The \([Ca^{2+}]_i\), of each cell was calculated according to formula by Grynkiewicz et al. (1985):

\[
[Ca^{2+}]_i = \frac{K_x \times b \times (R - R_{\text{min}})}{(R_{\text{max}} - R)}
\]

In the present study, \(K_x = 224 \text{ mM, } b = 21.5, R_{\text{max}} = 7.08\). These values were obtained by separately determining the 380-nm excitation fluorescence and the 340/380-nm excitation fluorescence ratio (R) in an "intraacellular" K+-rich medium that was Ca2+-deficient or saturated with Ca2+.

The selection of β cells for analysis was facilitated by the fact that 70–80% of the cells in the islet were demonstrated to be β cells by insulin-reactive immunocytochemical staining. Further identification of β cells was based on the larger size and low nuclear/cytoplasmic volume ratio relative to α and δ cells (Van De Winkel et al., 1982).

**Insulin secretion.** — To confirm that the secretory capacity of the β cells was retained, insulin secretion of dispersed islet cells (approximately 80 islets per assay) was determined at glucose concentrations identical to those used for \([Ca^{2+}]_i\), measurements. Islets obtained from the same rats as those used for the \([Ca^{2+}]_i\), measurements were dispersed and the cells suspended in 300 μl KRB buffer containing 5.5 mM glucose and 0.5% BSA. The cells were incubated at 37 °C.
for 20 min, after which time the medium was replaced with 300 μl KRB containing 7.5 mM glucose. The cells were then incubated for another 20 min. This process was repeated for two more 20-min incubation periods in which the medium glucose concentrations were 11.1 and 16.7 mM, respectively. At the end of each incubation period, a 100 μl aliquot was taken to determine insulin concentration by radioimmunoassay (Yalow et al., 1960). The total cell number was obtained by counting after the incubations. Because insulin secretion could not be evaluated in each individual cell, direct correlations made between the values for insulin secretion performed on groups of β cells (see Table 4), and values for [Ca2+]i (see Tables 2 and 3) measured on individual cells may not be appropriate.

Statistics. — Data from the measurement of [Ca2+]i were analyzed by using factorial analysis of variance (ANOVA). Two- and three-way designs were used where appropriate. One-way ANOVA was used to determine possible differences in the main effects. When a significant main effect was found, Fisher’s least significant difference post hoc test was used to evaluate possible differences. Differences were considered significant at p < .05.

The chi-square test was used to determine if β-cell responsiveness to glucose stimulation, defined as a change in [Ca2+]i, of at least 50% above the basal level [Ca2+]i (Ribes et al., 1981), was significantly associated with age and incubation medium glucose concentration. Differences were considered significant at p < .05.

In this study, the β cell, not the rat, was used as the experimental unit. We evaluated the possibility that β-cell populations obtained from different rats within each age group may differ with regard to responsiveness and sensitivity to glucose, a finding that would preclude the use of the β cell as the experimental unit. Two-way ANOVA indicated no significant effect of the animal on β-cell response.

RESULTS

Heterogeneity of β-cell responsiveness to glucose. — As the incubation medium glucose concentration was increased from 5.5 to 7.5, 11.1, and 16.7 mM, changes in [Ca2+]i among individual β cells were observed to be heterogeneous in both degree of responsiveness and in sensitivity to glucose. However, based on changes in [Ca2+]i, following glucose stimulation, the majority (~80%) of the β cells, regardless of the age of the animal from which they were isolated, could be placed into one of three categories: glucose-nonresponsive, responsive to 7.5 mM glucose, and responsive to 11.1 mM glucose. Figure 1 illustrates typical responses of [Ca2+]i by individual β cells exposed to an increasing concentration of glucose in the incubation medium. The pattern of response represented by line A exhibited an initial decrease in [Ca2+]i upon exposure to 7.5 mM glucose, followed by a rapid increase that was sustained at the higher glucose concentrations. The [Ca2+]i pattern represented by line B was similar to that of line A except that the rapid increase in [Ca2+]i did not occur until the glucose concentration of the medium was increased to 11.1 mM, the maximal glucose concentration for responsiveness observed in this investigation. Line C represents the pattern observed of the nonresponsive β cells with no significant change in [Ca2+]i upon exposure to any of the glucose concentrations.

Of all β cells examined (i.e., without regard to age), approximately 70% were responsive to a glucose stimulus. However, β-cell responsiveness was not evenly distributed among the age groups. The percentage of β cells that were nonresponsive to glucose at concentrations above 5.5 mM was significantly decreased in the 26-mo-old rats (p < .028) as compared to the 6- and 12-mo-old animals (Table 1). That is, more of the β cells of the 26-mo-old rats were able to respond to glucose by increasing [Ca2+]i, following glucose stimulation. Of all β cells that were glucose-responsive (Table 1), the percentages of cells initially responding at 7.5 and 11.1 mM glucose were not significantly different between the 6- and 12-mo-old animals. The 26-mo-old animals had a greater percentage of cells that initially responded at 7.5 mM glucose as compared to the two younger age groups (p < .019).

Table 1. Responsiveness to a Glucose Stimulus of Pancreatic β Cells Isolated From F344 Rats of Different Ages

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Nonresponsive Cells (% of all cells)</th>
<th>Responsive Cells (% of glucose-responsive cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (n = 84)</td>
<td>36.9*</td>
<td>57.5*</td>
</tr>
<tr>
<td>12 (n = 82)</td>
<td>35.4*</td>
<td>60.0*</td>
</tr>
<tr>
<td>26 (n = 125)</td>
<td>24.0*</td>
<td>72.4*</td>
</tr>
</tbody>
</table>

Notes: Individual β cells were grouped into three categories based on changes in [Ca2+]i following glucose stimulation: glucose-nonresponsive, responsive at 7.5 mM glucose, and responsive at 11.1 mM glucose. The criterion for responsiveness was a >50% increase in [Ca2+]i, over basal [Ca2+]i, at 5.5 mM glucose. Within a column, values not sharing a common letter superscript are significantly different by chi-square test (p < .05). n = number of cells analyzed.
Effects of age and glucose concentration on \([Ca^{2+}]_i\). — Two-way ANOVA indicated that there was no significant main effect of age on \([Ca^{2+}]_i\) at basal or stimulatory concentrations of glucose (Table 2). However, a significant main effect of incubation medium glucose concentration was observed \((p < .0001)\). After incubating for 20 min in the presence of 7.5 mM glucose, the average \([Ca^{2+}]_i\) of cells in each age group increased by at least 50% over the \([Ca^{2+}]_i\) at 5.5 mM glucose and was significantly different. Likewise, values for \([Ca^{2+}]_i\) at 11.1 mM glucose were significantly greater than those observed at 7.5 mM, as were values at 16.7 mM significantly greater than values at 11.1 mM.

The \([Ca^{2+}]_i\) values of nonresponsive \(\beta\) cells at the 5.5 mM glucose concentration were 66.7 ± 4.6, 57.4 ± 5.0, and 81.4 ± 4.7 nM for 6-, 12-, and 26-mo-old rats, respectively, and did not differ among the age groups or with increased glucose concentration. The values for \([Ca^{2+}]_i\) of nonresponsive \(\beta\) cells at 5.5 mM glucose were significantly less as compared to the values of responsive cells in each age group.

Further segregation of the \(\beta\) cells into categories of responsiveness at 7.5 or 11.1 mM glucose revealed marked differences in the way the cells responded (Table 3). Two-way ANOVA indicated no main effect of age at any incubation medium glucose concentration, although a main effect of glucose concentration was observed \((p < .0001)\). This main effect was attributed primarily to the increase in \([Ca^{2+}]_i\) of the 7.5 mM glucose-responsive \(7.5\)-responsive) cells relative to the 11.1 mM glucose-responsive \(11.1\)-responsive) cells. However, in general, the average \([Ca^{2+}]_i\) of the 7.5-responsive cells was significantly greater than that of the 11.1-responsive cells at the basal glucose concentration of 5.5 mM.

Effect of age on insulin secretion of the \(\beta\) cell. — The estimated average number of \(\beta\) cells per islet for the 6-, 12-, and 26-mo-old rats was 773 ± 90, 733 ± 278, and 642 ± 84, respectively, and did not differ significantly. When adjusted to reflect only the \(\beta\) cells that were responsive to glucose, the number of \(\beta\) cells per islet was nearly identical among the age groups at 487 ± 57, 476 ± 181, and 488 ± 64 for the 6-, 12-, and 26-mo-old animals, respectively.

These values were used in calculating \(\beta\)-cell insulin secretion, expressed as femtomoles/\(\beta\) cell/20 min (Table 4). Two-way ANOVA indicated no significant main effects of age at any of the incubation medium glucose concentrations. However, a significant main effect of incubation medium glucose concentration \((p < .009)\) was observed. This main effect was attributed primarily to the increase in insulin secretion at 7.5 mM as compared to the basal glucose concentration of 5.5 mM.

**DISCUSSION**

A goal of the present investigation was to evaluate a possible mechanism by which some organs or physiological systems are able to resist age-related decrements in function. Using the pancreatic \(\beta\) cell as a model for tissues that appear to retain youthful activity with age, we have shown that a significant shift in the balance of both responsiveness and sensitivity to glucose occurs among cells of 26-mo-old F344 rats as compared to 6- and 12-mo-old animals (Table 1). The percentage of \(\beta\) cells that were glucose-responsive was significantly greater in the 26-mo-old rats as compared to the 6- and 12-mo-old animals. Of the glucose-responsive cells, a significantly greater percentage of those from the oldest age group were more sensitive to glucose (i.e., responded at the lower glucose concentration) as compared to the two younger age groups. Thus, these data support the hypothesis that the maintenance of insulin secretory capacity in the senescent F344 rat is accomplished, in part, through alterations in the functional heterogeneity of the \(\beta\)-cell population.

The apparent shift in functional heterogeneity of the \(\beta\) cells, isolated from older vs younger rats, may be related to the decrease in the total number of \(\beta\) cells in the aging islet of...
Langerhans. That is, we found that the total number of β cells within islets of similar size isolated from the senescent rat was generally less than that observed in islets from younger animals. However, when the number of β cells per islet was adjusted to reflect only the percentage of cells that were glucose-responsive, the absolute number of cells that could respond to glucose stimulation was nearly identical across the age groups. Thus, it appears that the end result of the age-related increase in the percentage of β cells that are glucose-responsive is that an equal number of β cells secrete an equal amount of insulin in all age groups. Considering the fact that decreased cellular proliferation is a general hallmark of aging (Hayflick, 1985), it is possible that the endocrine pancreas compensates for age-related diminution of function caused by decreases in cell number by increasing the responsiveness and sensitivity of the cells that remain.

The results of the present investigation verify the findings of others that β cells can be divided into glucose-responsive and glucose-nonresponsive populations. Our findings in aging rats are in agreement with previous reports using younger animals describing a population of β cells that remain inactive with respect to insulin release (Schantz et al., 1988; Pipeleers, 1992; Van Schravendijk et al., 1992; Wang et al., 1992). Reasons for the presence of a relatively large subpopulation of glucose-nonresponsive cells within a tissue that exists primarily to respond to an increased plasma glucose concentration are not immediately apparent. It has been proposed that some or all of the cells within this population may be at rest (Hirahata and Ramirez-Mendez, 1991) or may be involved in cell replication (Swenne, 1992). It is also possible that the nonresponsive cells have reached the end of their life span. This is unlikely, however, as our pilot studies (see Materials and Methods) have shown that β cells isolated from older rats are viable with respect to membrane integrity. In addition, others have shown that the glucose-nonresponsive β cells are able to secrete insulin under certain conditions (Van Schravendijk et al., 1992; Wang et al., 1992, 1993). Another possibility is that the glucose-nonresponsive β cells represent cells that respond to insulinotropic agents other than glucose. The existence of such β cells has been demonstrated by Wang et al. (1992), who showed that the acetylcholine-stimulated increase in [Ca2+]i, that occurs in most nonresponsive β cells was potentiated by high glucose concentrations in some of these cells but not in others. Similar results have been obtained with other insulinotropic agents, including glucagon (Wang et al., 1993), cholecystokinin-8 (Wang et al., 1992), glyburide (Draznin et al., 1985; Rojas et al., 1994), and muscarine (Rojas et al., 1994). The existence of β cells that respond to agents other than glucose may allow more flexibility in the regulation of insulin secretion by responding to insulinotropic agents secreted during physiologic states separate from, or in conjunction with, an increase in plasma glucose concentration. Nonetheless, the precise function of these glucose-nonresponsive β cells is not well understood, and the effects of age on their activity are even less clear.

The results of the present study indicated that the glucose-stimulated increase in [Ca2+]i of the β cell did not change with age and thus appeared unrelated to the observed alterations in functional heterogeneity. However, the relationship among aging, calcium regulation, and β-cell responsiveness/sensitivity to glucose is complex and is affected by several factors intrinsic and extrinsic to the β cell. It is possible that changes in the physiologic or metabolic milieu of the senescent animal may elicit a shift in the quantity and/or efficacy of one or more of these regulatory factors. The resultant change in activity of these calcium-modulating factors may correspond to the age-related shift in glucose responsiveness/sensitivity of the β-cell population. For example, cAMP and protein kinase C (PKC) are intracellular potentiators of glucose-stimulated insulin secretion and are associated with the regulation of [Ca2+]i. Considerable evidence suggests that cAMP and PKC modulate calcium channel activity to increase glucose-stimulated calcium flux, resulting in the potentiation of insulin secretion (Hubinont et al., 1980; Malaisse and Malaisse-Lagae, 1984; Henquin, 1985; Malaisse et al., 1985). It has also been shown that the increase in [Ca2+]i, and thus the responsiveness of β cells to glucose stimulation, is increased by glucagon, an agent that elevates intracellular cAMP levels, and acetylcholine, an activator of PKC (Wang et al., 1993). Any age-related change in the production of, or sensitivity to, cAMP or PKC could result in altered β-cell responsiveness to glucose. However, to date there is a paucity of information regarding the effects of age on the secretion of glucagon, acetylcholine, and other agents that affect [Ca2+]i and insulin secretion.

The study of mechanisms accounting for aging has been, to a large degree, investigated by comparing a specific outcome variable (i.e., survival, free radical damage, glycation of proteins, etc.) between different age groups (e.g., young vs old). The study of these outcomes in populations is complicated by the fact that organisms and/or cells that do not follow the pattern of the aggregate population are either eliminated from the study (i.e., outliers) or are lost in the "average." Such an approach to data analysis poses few problems in younger populations in which the variance about the mean between the groups is relatively constant and is most likely due to experimental error rather than organism-to-organism variance. On the other hand, the increased heterogeneity of variance that is well characterized in older populations is due to differences in the rate of aging between individuals or cells (Rogers, 1991). The data presented here are consistent with these observations in that we have shown considerable variation in responsiveness and sensitivity to glucose concentration by incubation medium glucose concentration (p < .003).
glucose between young and old β cells. These findings suggest that experimental design in aging studies should incorporate measures of individual differences rather than focus exclusively on differences between means of groups.

In summary, the results presented here indicate that in the rat, the maintenance of organ function during senescence at a level comparable to that of younger animals may be accomplished, in part, by an increase in the percentage of cells that are responsive to stimuli and/or by an increase in the sensitivity of the responsive cells. In the present investigation, the increase in glucose responsiveness of the β-cell population isolated from the aging animal did not reflect alterations in the glucose-stimulated increase in [Ca^{2+}]. Although the mechanism underlying the age-related increase in β-cell responsiveness/sensitivity to glucose has yet to be elucidated, it is possible that alteration in heterogeneity of function is a common feature among organ cell populations. Such alterations, in turn, may permit the maintenance of organ function within the changing metabolic and physiologic milieu of the senescent animal.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (AG-00429) and gifts from The Sugar Association Inc. and the California Age Research Institute.

The authors wish to acknowledge Carol Murtagh-Mark for her technical support and Dr. Charles Plopper for his helpful advice and for providing access to his calcium imaging system.

Address correspondence to Dr. Roger McDonald, Department of Nutrition, University of California, Davis, CA 95616. E-mail: rmcmcdonald@ucdavis.edu

REFERENCES


Received September 27, 1995

Accepted January 18, 1996