The Nature of Insulin Secretory Defect in Aging Rats

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SUMMARY

We have attempted to define the nature of insulin secretory defect(s) in aged animals. In these studies, pancreatic islets were isolated from 2- and 18-mo-old Fischer 344 rats. Margination of secretion vesicles during exocytosis was assessed by measuring the recruitment of somatostatin (SRIF) receptors to the surface membrane. Section vesicle lysis was studied by measuring insulin release into the incubation media.

Submaximal and maximal glucose-induced insulin secretion was significantly greater in islets isolated from younger rats ($P < 0.01$). SRIF receptor recruitment was stimulated by glucose in both younger and older Fischer 344 rats. However, an increase in SRIF receptor recruitment was reduced in islets isolated from older animals (from 2.14 ± 0.4 to 4.6 ± 0.4 fmol/10 islets) ($P < 0.01$) as compared with islets from younger animals (from 2.6 ± 0.2 to 6.2 ± 0.4 fmol/10 islets). When secretion vesicle lysis was inhibited by the presence of sodium isethionate in the incubation media, glucose (300 mg/dl) failed to stimulate secretion vesicle margination to the plasma membrane. In contrast, glyburide (0.6 μg/ml) continued to stimulate directly secretion vesicle margination in islets from aged animals (from 2.1 ± 0.3 to 6.0 ± 0.3 fmol/10 islets).

We conclude that glucose-induced margination of secretion vesicles at the plasma membrane is impaired by the aging process. This impairment results in lower submaximal and maximal insulin secretory response to glucose. The fact that glyburide is capable of stimulating secretion vesicle margination suggests that glucose signal recognition and/or stimulus-secretion coupling may be the locus of impairment in the process of insulin secretion in older animals. DIABETES 1985; 34:1168–73.

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Previous studies[1–3] demonstrated that maximal glucose-stimulated insulin secretion by pancreatic islets of aging Sprague-Dawley (18 mo old) and Fischer 344 rats (12 mo old) is reduced when compared with the response of islets isolated from younger rats (2 mo old). Decreased insulin secretory responses to glucose have been observed in experiments in isolated islets and the intact perfused pancreas when results were calculated in terms of islet insulin content.[1,4,5] Neither obesity nor caloric restriction had significant influence on changes in the insulin secretory response of islets isolated from aging animals.[4] The decline in B-cell response to glucose was attributed primarily to the aging process per se.

In contrast to the decreased glucose-stimulated insulin release, the total islet insulin content was found to be increased in older animals.[3,6] This suggested that the age-related defect in insulin secretion may involve one or several steps in the process of exocytosis: signal recognition, stimulus-secretion coupling, the migration of secretion vesicles to the plasma membrane, their fusion with the plasma membrane, and subsequent lysis.

We have recently developed a method for studying separately the process of secretion vesicle migration to the plasma membrane and secretion vesicle lysis.[7,8] The surface membrane somatostatin (SRIF) receptor concentration is enhanced concomitant with secretion vesicle fusion to the plasma membrane. One can ascertain the event of margination of secretion vesicles at the plasma membrane by measuring the recruitment of SRIF receptors during exocytosis. We define margination as the fusion of secretion vesicles with the plasma membrane. The subsequent lysis of marginated granules is documented by measuring the release of insulin into the surrounding media. Using this approach, we have shown that glucose stimulates both secretion vesicle margination and secretion vesicle lysis.[9]

In the present study, we attempted to determine the nature

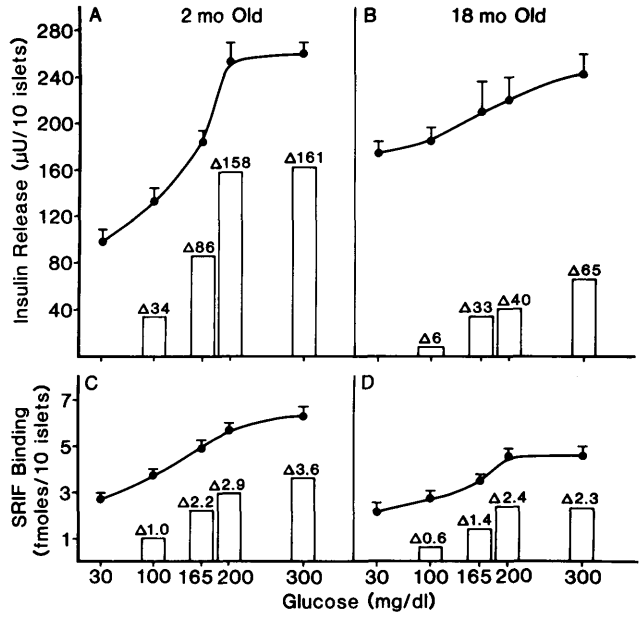

FIGURE 1. Effect of glucose on insulin release and SRIF receptor recruitment in pancreatic islets isolated from 2-mo-old (A, C) and 18-mo-old (B, D) Fischer 344 rats. Incubations were carried out for 30 min at 37°C. Results represent mean ± SEM of six experiments. Bars indicate the increments above the basal levels (glucose 30 mg/dl).

of the insulin secretion defect in aged animals—whether it involved secretion vesicle margination or lysis or both. Fischer 344 rats were chosen because weight gain is not as prominent as in Sprague-Dawley rats at any given age. Thus aging alone rather than obesity and inactivity might be considered responsible for the changes observed.

MATERIALS AND METHODS

Animals. Fischer 344 rats (2 mo and 18 mo old) were obtained from the National Institute of Aging, Bethesda, Maryland. The animals were allowed food and water ad libitum. Anesthesia was induced with sodium pentobarbital (45 mg/kg body wt).

Materials. Collagenase CLS-IV (126 U/mg) was obtained from the Worthington Biochemical Corporation, Freehold, New Jersey. ^{125}I-insulin was purchased from Cambridge Medical Diagnostics, Inc., Billerica, Massachusetts, and ^{125}I-somatostatin from the New England Nuclear Corporation, Boston, Massachusetts. Unlabeled insulin was a gift from Dr. R. Chance, Eli Lilly and Company, Indianapolis, Indiana, and glyburide was supplied by the Upjohn Co., Kalamazoo, Michigan. Isobutylmethylxanthine (IBMX) and sodium isethionate (NaIs) were obtained from the Sigma Chemical Company, St. Louis, Missouri.

Isolation of islets. Pancreatic islets were prepared from male, 2- and 18-mo-old Fischer 344 rats by the method of Lacy and Kostianovsky,[10] with modifications as previously described.[11] After isolation, the islets were kept in Krebs-Ringer bicarbonate buffer containing 30 mg/dl glucose and 2 mg/ml bovine serum albumin (BSA). The buffer had previously been gassed with 95% O_2/5% CO_2 for 30 min and adjusted to pH 7.4. In the experiments using NaIs, this agent was substituted for NaCl in equimolar concentrations (120 mM).

Incubation protocols for insulin release and SRIF binding. After preincubation, 10 islets were transferred into a series of 12 × 75-mm glass tubes containing 200 μl gassed Krebs-Ringer buffer with 30 mg/dl glucose and 1 mg/ml BSA at 2°C. This was followed by an addition of 800 μl gassed Krebs-Ringer bicarbonate buffer containing sufficient amounts of secretagogue to yield final concentrations of 30, 100, 165, 200, or 300 mg/dl glucose; 0.6 μg/ml glyburide; or 400 μM IBMX. The sample tubes were then incubated at 37°C for either 10 or 30 min and aliquots of medium were carefully removed from each tube for determination of insulin release by double-antibody radioimmunoassay.[12] The islets in the remaining media were used for SRIF binding studies. Binding experiments were performed as described previously.[7-9,11] In brief, a buffered medium (200 μl) consisting of 25 mM ethylenediaminetetraacetic acid, 0.1% BSA, and 500 kallikrein-inactivating U/ml Trasylol in 50 mM Tris, pH 8.0, is added to the 200 μl of medium containing the incubated islets. Finally, 15 μl of label containing 2 ng of ^{125}I-SRIF (250,000 cpm) is introduced into the incubation medium. In all studies, samples are prepared in triplicate. Three "total-count" tubes are included in each incubation. All preparatory steps are carried out at 2°C and binding is determined by incubation of the samples at 4°C for 18 h.

The binding reactions are terminated by adding ice-cold ethanol to the medium to yield a final concentration of 85% vol/vol. The samples are allowed to stand at 2°C for a period of 10 min and then centrifuged at 1800 × *g* for 10 min at 4°C. The entire supernatant is carefully removed and discarded. Pellet radioactivity is determined in a Searle Analytic 1185 automatic gamma-counting system (Searle Radiographics, Inc., Des Plaines, Illinois).

Specific binding is determined as the difference between

TABLE 1
Islet size and insulin release in 2- and 18-mo-old Fischer 344 rats

	Islet diameter*		Basal IR†		Glucose-stimulated IR‡	
Fischer 344 rats	N§	(μm)	μU/10 islets	μU/μm	μU/10 islets	μU/μm
2 Mo old	30	168 ± 8	99 ± 8	0.059	256 ± 10	0.152
18 Mo old	30	276 ± 10	170 ± 10	0.061	243 ± 15	0.088

*Diameter was measured with micrometer fitted into the ocular of a dissecting microscope.
†IR, insulin release.
‡Glucose 300 mg/dl.
§Number of islets.

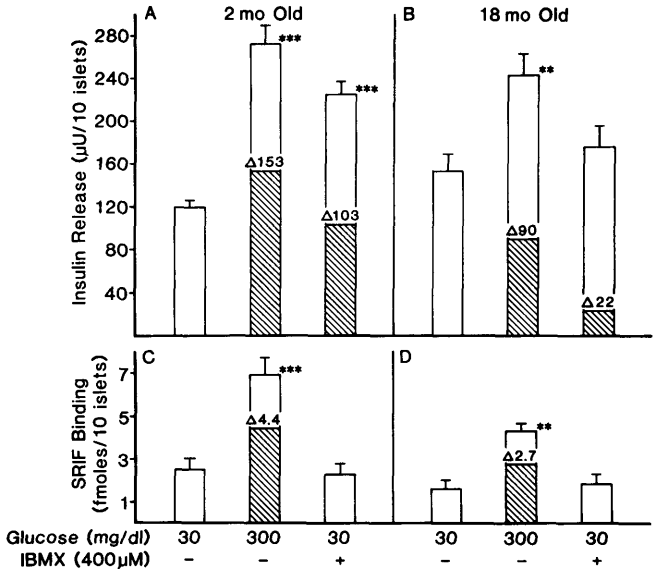

FIGURE 2. Effect of IBMX (10 min incubation) on insulin release (A, B) and SRIF receptor recruitment (C, D) in islets isolated from 2- and 18-mo-old Fischer 344 rats. In control experiments the islets were incubated with either 30 or 300 mg/dl glucose. Results represent mean ± SEM of four experiments. Shaded areas in this and subsequent figures represent increments over the basal rate of insulin release and SRIF binding. Double asterisks (**) represent $P < 0.01$ versus glucose 30 mg/dl and triple asterisks (***) $P < 0.001$ versus glucose 30 mg/dl.

the samples containing 0 and 20 ng/ml unlabeled SRIF, these representing total binding and nonspecific binding, respectively.

Paired or unpaired Student's *t*-test was used to compare the mean values in different experiments as appropriate.

RESULTS

In our initial experiments, we compared insulin release and recruitment of SRIF receptors in response to increasing concentrations of glucose in inslets isolated from 2- and 18-mo-old Fischer 344 rats. The results of these experiments are shown in Figure 1. Glucose prompted a dose-related increase in insulin secretion in the islets from younger animals (Figure 1A) with maximal stimulation being observed at glucose concentration of 200 mg/dl. In older animals (Figure 1B), the basal level of islet insulin secretion was increased ($P < 0.01$). Similar to the younger rats, the islets from older animals demonstrated dose-related, glucose-induced insulin release. However, the increments in insulin release over the basal nonstimulated level were significantly smaller in older animals than in younger ones. Because the islets isolated from 18-mo-old rats are larger than those isolated from younger animals, it is appropriate to compare insulin release per unit of islet size. The results of this comparison are summarized in Table 1. As can be seen, when expressed per unit of islet size, basal insulin release is identical in younger and older rats, whereas stimulated insulin release in older animals was approximately one-half that found in younger ones.

The recruitment of SRIF receptors in younger animals is shown in Figure 1C. After glucose-induced insulin release, SRIF binding rose from 2.6 ± 0.2 fmol/10 islets to a maximum of 6.2 ± 0.4 fmol/10 islets ($P < 0.001$). In older animals (Figure 1D), SRIF binding was slightly lower in the nonstimulated state (2.1 ± 0.4) but rose progressively in response to glucose stimulation to 4.6 ± 0.4 fmol/10 islets ($P < 0.01$). The recruitment of SRIF receptors in older animals at all glucose concentrations was of lesser degree than that observed in younger rats.

In the 2-mo-old Fischer 344 rats, both IBMX-induced (400 μM) and glucose-induced (300 mg/dl) insulin release were examined. In experiments with glucose (10 min incubation), insulin release was prompt (Figure 2A), as was the recruitment of SRIF receptors (Figure 2C). IBMX elicited rapid insulin release but no recruitment of SRIF receptors was seen within 10 min of incubation. In the older Fischer 344 rats (18 mo old), both glucose and IBMX stimulated insulin release of a lesser degree than that observed in younger animals. Similarly, glucose-induced recruitment of SRIF receptors was not as prominent as that seen in younger animals. Ten minutes incubation with IBMX resulted in no change in SRIF binding in islets isolated from older animals.

When the incubation time was extended to 30 min, we observed that both glucose (300 mg/dl) and IBMX (400 μM) produced insulin release and recruitment of SRIF receptors (Figure 3, A and C). The magnitude of stimulation of insulin release from islets isolated from older animals was considerably less after either glucose or IBMX stimulation. Similarly, recruitment of SRIF receptors in these older animals was of a lesser degree than that found in the younger animals.

We then examined the effect of the sulfonylurea agent glyburide on insulin secretion in islets isolated from 2- and 18-mo-old Fischer 344 rats. The results of these experiments are shown in Figure 4. In the islets isolated from the younger animals, both glucose (300 mg/dl) and glyburide (0.6 μg/ml) induced prompt release of insulin. While glucose-stim-

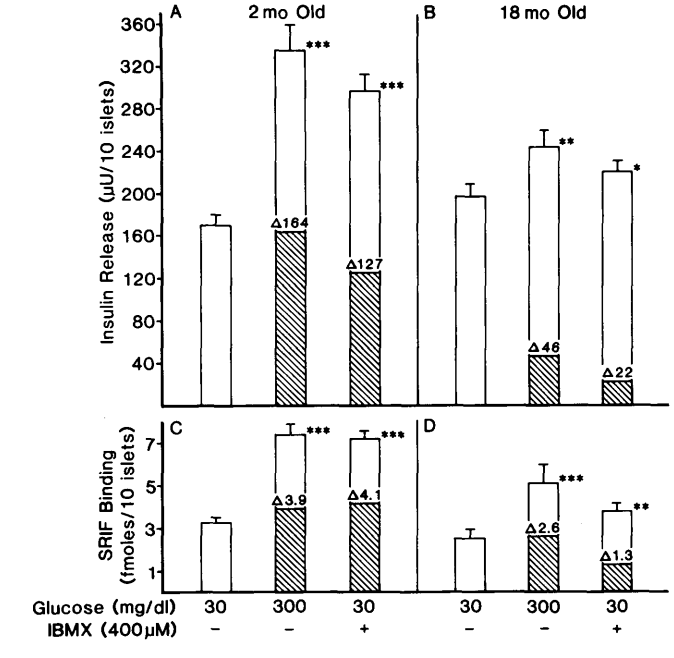

FIGURE 3. Effect of IBMX (30 min incubation) on insulin release (A, B) and SRIF receptor recruitment (C, D) in islets isolated from younger and older rats. In control experiments, the islets were incubated with either 30 or 300 mg/dl glucose. Results represent mean ± SEM of four experiments. Single asterisk (*) represents $P < 0.05$, double asterisks (**) $P < 0.01$, and triple asterisks (***) $P < 0.001$.

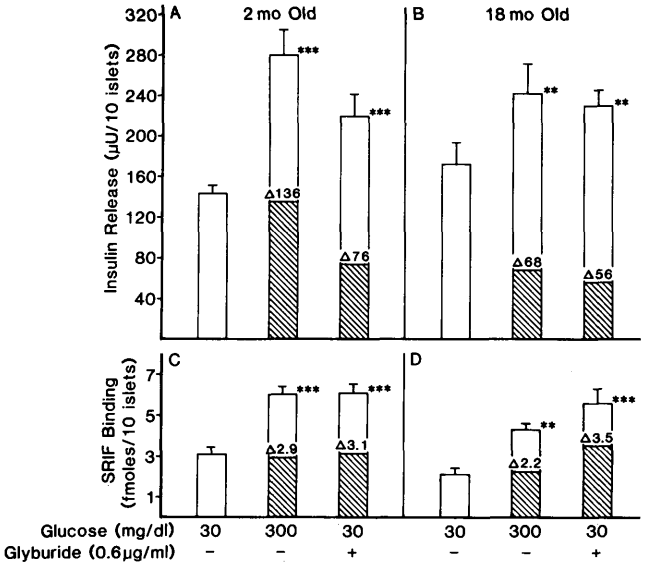

FIGURE 4. Effect of glyburide on insulin release (A, B) and SRIF receptor recruitment (C, D) in islets isolated from younger and older Fischer 344 rats. Incubations were carried for 30 min at 37°C. Control experiments were conducted with either 30 or 300 mg/dl glucose. Results represent mean ± SEM of six experiments. Single asterisk (*) represents $P < 0.05$, double asterisks (**) $P < 0.01$, and triple asterisks (***) $P < 0.001$.

ulated insulin secretion in the older animals was not as great as in the younger ones, glyburide-induced insulin release was almost identical in both younger and older animals. Incubation with either glyburide or glucose resulted in recruitment of SRIF receptors in islets isolated from younger animals. However, glucose-induced recruitment of SRIF receptors in the 18-mo-old animals was less than that seen in 2-mo-old rats. In contrast, glyburide-induced recruitment of SRIF receptors in older animals was identical to that observed in younger ones.

To explore further the effect of glyburide on insulin release in older animals, we incubated pancreatic islets with and without sodium isethionate, an inhibitor of secretion granule lysis.[7,13,14] The results of these experiments are shown in Figure 5. The presence of 120 mM sodium isethionate (substituted for NaCl) significantly reduced ($P < 0.01$) both glucose-induced and glyburide-induced insulin release in younger and older animals. In the younger animals, both glucose and glyburide continued to enhance recruitment of SRIF receptors even in the presence of sodium isethionate (Figure 5C) when secretion vesicle lysis was inhibited. In the older animals (Figure 5D), in the presence of sodium isethionate, glucose failed to enhance recruitment of SRIF receptors, whereas glyburide continued to stimulate migration of secretion vesicles to the plasma membrane.

DISCUSSION

The major goal of this study was to localize the impairment in insulin release from pancreatic islets isolated from aged animals. The results of these experiments indicate that aging may be associated with changes in the process of glucose-induced secretion vesicle migration and margination to the plasma membrane.

We have previously shown that glucose directly stimulates secretion vesicle margination.[9] The present experiments demonstrate that glucose-induced recruitment in SRIF receptors in older animals is impaired compared with that in younger animals. This became particularly evident in the experiments with sodium isethionate. Sodium isethionate inhibits lysis of secretion vesicles and insulin release[7-9] but does not interfere with secretion vesicle margination to the plasma membrane. The process of secretion vesicle margination at the plasma membrane is reflected by an enhancement in recruitment of SRIF receptors to the plasma membrane. In the presence of sodium isethionate, glucose directly stimulates secretion vesicle margination to the plasma membrane both in young Sprague-Dawley[9] and Fischer 344 rats (present study). In contrast, glucose failed to marginate secretion vesicles in the older animals, suggesting a defect in the ability of pancreatic islets of older animals to translocate secretion vesicles to the plasma membrane.

In contrast to glucose, glyburide continued to stimulate secretion vesicle migration and margination at the plasma membrane in older rats. Although the precise mechanism of glyburide action remains unknown, we have previously shown that it can stimulate directly secretion vesicle migration and secretion vesicle lysis.[9] This action of glyburide is independent of extracellular calcium, whereas glucose-induced migration of secretion vesicles is dependent on the presence of extracellular calcium.[9] The present experiments demonstrate another distinction between glucose- and glyburide-induced insulin release. Whereas pancreatic islets isolated from older animals are unresponsive to glucose in terms of stimulating secretion vesicle migration, the islets remain sensitive to glyburide.

Recently, Molina et al.[15] have reported a somewhat similar difference between glucose and glyceraldehyde in terms of their effect on insulin release from islets isolated from older animals. The fact that glyceraldehyde elicited insulin release when glucose failed to do so indicates that the pathway per

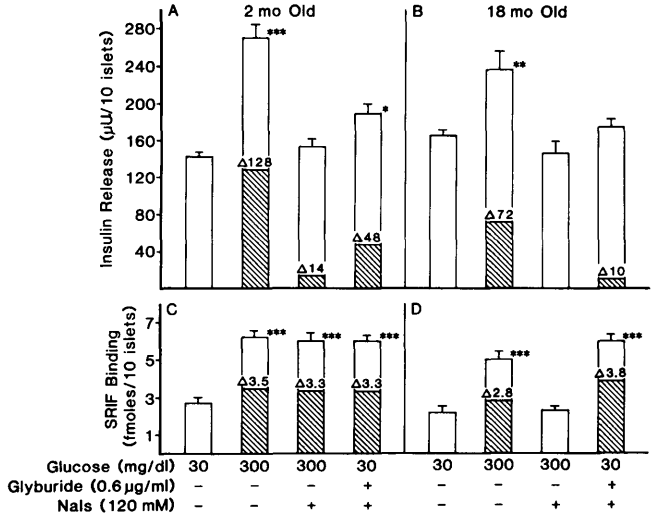

FIGURE 5. Effect of glucose and glyburide on secretion vesicle margination in the islets isolated from 2- and 18-mo-old Fischer 344 rats. Panels A and B represent insulin release while panels C and D demonstrate SRIF receptor recruitment. Experiments were conducted with and without Nals (120 mM) and carried out for 30 min at 37°C. Results represent mean ± SEM of six experiments. Single asterisk (*) represents $P < 0.05$, double asterisks (**) $P < 0.01$, and triple asterisks (***) $P < 0.001$.

se is likely to be intact but glucose signal recognition is impaired.

Incubation of the islets for 10 min with IBMX (an agent that induces lysis of the secretion vesicles) resulted in prompt release of insulin in younger animals and a diminished response in older animals. In a previous study,[16] we have shown that IBMX-induced acute insulin release is highly correlated with the concentration of the secretion vesicles marginated at the plasma membrane. It seems likely that the decreased insulin response to IBMX in older animals (secretion vesicle lysis) reflects a diminished number of marginated secretion vesicles. The results however do not discount the possibility of an additional impairment of the granule lytic process in aged animals, although this seems less likely.

We have suggested[9] that the margination of secretion vesicles to the plasma membrane appears to occur as the result of two somewhat independent processes: (1) a rapid secretagogue-stimulated margination and fusion of secretion vesicles with the plasma membrane and (2) delayed margination, which occurs secondary to the lysis of premarginated secretion vesicles. Whereas the rapid margination process appears to be calcium dependent, the delayed, passive lysis-driven margination can occur in the absence of extracellular calcium.[9]

In Sprague-Dawley[9] and young Fischer 344 rats (present study), glucose had stimulated SRIF receptor recruitment in islets incubated with sodium isethionate (i.e., when secretion vesicle lysis is inhibited). In islets isolated from older Fischer 344 rats, glucose failed to promote SRIF receptor recruitment in the presence of sodium isethionate (Figure 5D). Taken together, these data indicate that this recruitment of SRIF receptors in aged animals occurs only secondary to the lysis of previously marginated vesicles. This delayed lysis-driven margination of secretion vesicles is also observed in experiments with IBMX. IBMX enhanced SRIF receptor recruitment after 30 min (Figure 3) and not after 10 min (Figure 2) of incubation. This delayed recruitment of SRIF receptors was inhibited in the presence of sodium isethionate, when the lysis of secretion vesicles was blocked.

Our results suggest that in older animals, the major component in exocytosis contributing to granule fusion at the plasma membrane is delayed margination of secretion vesicles. Nevertheless, the experiments with IBMX (30 min incubation, Figure 3) demonstrate some degree of impairment in this delayed margination pathway (i.e., the magnitude of SRIF receptor recruitment in islets isolated from older animals in significantly smaller than in islets isolated from younger rats).

Currey et al.[3] have recently shown that both submaximal and maximal glucose-induced insulin secretion in the perfused rat pancreas were decreased in 12-mo-old Fischer 344 rats. They observed, however, that the magnitude of the age-related reduction in glucose-induced insulin release was less with submaximal than with maximal glucose stimulation. Our results in isolated islets are in agreement with those of Currey et al. (obtained in perfused pancreata),[3] indicating that both submaximal and maximal glucose-induced insulin release is diminished in 18-mo-old Fischer 344 rats. Our experiments do not indicate that maximal glucose-induced insulin secretion is more impaired than submaximal stimulation.

The results of the present experiments also demonstrate the enhanced basal insulin secretion in older animals. This was accompanied by lower SRIF binding to unstimulated islets. One explanation for the enhanced basal insulin secretion in older rats could be the greater volume of pancreatic islets in aged animals. Curry et al.[3] have calculated insulin secretion per islet cell mass. They observed that although the absolute amount of insulin released from islets isolated from older animals is greater, insulin secretion per islet cell mass was smaller in 12-mo-old rats than in 2-mo-old animals.

When the present data are calculated per unit of islet size (Table 1), basal insulin secretion is not increased in aged animals. It is important to realize that although insulin secretion per unit size is identical (basal) or smaller (stimulated) in older animals, the absolute amount of insulin secreted is either greater (basal) or just slightly below (stimulated) that observed in younger counterparts. Both insulin release per unit of islet size and an absolute amount of insulin secreted are important variables in understanding the physiology of insulin secretion in aging. Underestimation of either one can lead to erroneous conclusions.

Our interpretation of the data presented here is based on the assumption that alterations in SRIF binding signify margination of the secretion vesicles. Although we have previously presented ample evidence in favor of this assumption,[7–9,11] we cannot completely exclude the possibility that there might be other intracellular binding sites for SRIF that migrate to the plasma membrane during exocytosis.

Taken together, these results strongly suggest an impairment in the ability of islets isolated from older animals to marginate secretion vesicles to the plasma membrane in response to glucose stimulation. The margination is however sensitive to glyburide, indicating that this process per se is intact but recognition of glucose or glucose-triggered stimulus-secretion coupling is impaired.

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