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Impaired insulin release in aging rats: metabolic and ionic events

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Abstract. We investigated the effect of aging on glucose uptake, glucose-induced O_2 consumption, glucose-induced ^{45}Ca movements, and calmodulin content to elucidate age-related impairment of glucose-induced insulin release in pancreatic islets of Wistar rats. Intact pancreatic islets from old (24-month-old) rats showed impaired glucose-induced insulin release; glucose uptake and O_2 consumption were lower in old than in young (2-month-old) or adult (12-month-old) rats. Moreover, ^{45}Ca uptake and calmodulin content were decreased in pancreatic islets from older rats, which explained the impairment in glucose-induced insulin release in aging. No major differences between the 3 age groups in glucose-induced ^{45}Ca efflux in pancreatic islets were observed.
Key words. Aging; glucose uptake; calmodulin; insulin release; pancreatic islets.

We have previously shown that glucose-stimulated insulin release in perifused pancreatic islets is reduced in aging rats (24-month-old) as compared to young (2-month-old) animals[1]. Recently we reported that rapid weight loss due to caloric restriction does not significantly affect the impaired pattern of glucose-induced insulin release in aging rats as compared to adult (12-month-old) or young (2-month-old) rats[2]. Decreased glucose-induced insulin release is therefore an intrinsic feature of the aging process, which is in accordance with other studies[3,4]. However, the total islet insulin content is increased in older animals[5], suggesting that the age-related defect in insulin secretion may involve one or more steps of the insulin secretion process.

Previous studies have suggested that glucose-induced insulin release in the pancreatic islets of young rats is related to pentosephosphate shunt activity[6], to the redox state of reduced pyridine nucleotides, and to reduced glutathione[7]. However it has been shown that diminished glucose-induced insulin release from pancreatic islets in old rats is not due to a decrease redox state of the NAD(P)H-NAD(P) system. According to Ammon et al.[8] the failure of glucose to increase reduced glutathione formation in pancreatic islets of older animals may be responsible in part for the decreased secretory response of pancreatic islets in old rats. In addition, the activity of α-glycerophosphate dehydrogenase, key enzyme of the glycerophosphate shuttle system, is reduced, leading to the expected changes in pyridine nucleotide content, both in the basal state and in response to elevated glucose levels[9]. On the basis of these results it is reasonable to suggest that the age-related decline in glucose-stimulated insulin secretion per β-cell is secondary to a reduction in rate of glycolysis in β-cells of older animals[9].

The aim of the present study was to investigate the effect of aging on some steps involved in glucose-induced insulin secretion, including glucose uptake as the first signal for insulin release, and O_2 consumption as an indicator of metabolic activity in pancreatic islets from old rats. We also studied ^{45}Ca movements and calmodulin content as steps involved in stimulus-secretion coupling.

Materials and methods

Animals. Pancreatic islets were isolated from 2-, 12- and 24-month-old male Wistar rats. The animals had unlimited access to water and standard chow, and were maintained on a 12-h light-dark cycle.

Incubation, washing, and perifusion medium. The medium used for incubation, washing and perifusion was a modified Krebs-Henseleit bicarbonate buffer containing glucose 2.7 or 16.7 mM, Na^+ 145 mM, K^+ 5.0 mM, Cl^- 126 mM, Mg^{2+} 0.7 mM, Ca^{2+} 1.2 mM, HCO_3^- 25 mM, SO_4^{2-} 0.7 mM, and $H_2PO_4^-$ 3.6 mM supplemented with 10 mM HEPES and 0.5% bovine albumin, and adjusted to pH 7.4.

Isolation of intact pancreatic islets and measurement of glucose uptake. Intact pancreatic islets were isolated by a modification of the collagenase (Boehringer 2200 U/mg) method[10]. Briefly, the pancreas was distended by passing 12 ml ice-cold Hanks saline (pH 7.4) containing collagenase 0.76 mg/ml (Collagenase P Boehringer) along the pancreatic duct. The pancreas was excised and then shaken (100 cycles/min) at 37 °C for 20–30 min. The partially disaggregated tissue was suspended in 10 ml ice-cold Hanks saline and drawn up and expelled 6–10 times through a 6.5 cm 14-gauge hypodermic syringe needle until a homogenous suspension was obtained. This was centrifuged for 10 s at 200 g; the pelleted material was subjected to two more cycles of

resuspension and centrifugation and then filtered through a 6.5 cm plastic net strainer (0.5 mm mesh). Each filtrate was centrifuged for 10 s at 200 g and the pellet was resuspended in 4 ml Histopaque 1119 (Sigma) in Universal tubes overlayered with 4 ml Histopaque 1077 followed by 4 ml Hanks solution. The tube was centrifuged for 25 min at 500 g and isolated pancreatic islets were recovered from the saline/Histopaque 1077 interface and washed twice in incubation medium.

After a 30 min preincubation period, batches of 10 islets were incubated for 2 min in 0.5 ml incubation medium containing 1-[^{14}C]glucose (2 µCi/ml) at a final concentration of 16.7 mM; 0.3 ml silicone followed by 0.5 ml SDS-OHNa solution were placed under the incubation medium. After incubation, the tubes were centrifuged at 2000 g for 1.0 min. The SDS-OHNa solution containing pancreatic islets was added to 10 ml of scintillation liquid.

O_2 consumption assay. After a preincubation period, 40 pancreatic islets were incubated in incubation medium with glucose at 2.7 mM or 16.7 mM. The O_2 consumption was measured with a noncommercial clark-type selective electrode.

^{45}Ca uptake. Incubation medium (0.5 ml), containing 10 pancreatic islets and ^{45}Ca (20 µCi/ml) at a final concentration of 1.2 mM, 0.3 ml silicone, and 0.5 ml of SDS-OHNa solution were placed in microfuge tubes, incubated for 5 min and immediately centrifuged at 2000 g for 1 min. ^{45}Ca uptake was assayed by counting scintillations from the SDS-OHNa solution mixed with 10 ml of scintillation liquid.

Insulin release and ^{45}Ca efflux. Batches of 100 intact pancreatic islets were incubated for 60 min in the presence of ^{45}Ca (20 µCi/ml) and glucose (16.7 mM).

After incubation, the islets were washed 5 times with free ^{45}Ca medium containing 1 mM EGTA and placed in a perifusion chamber connected to two reservoirs through a Y-shaped valve. The perifusate was delivered at a constant rate (1.0 ml/min). Immunoreactive insulin (IRI) was measured in an aliquot of perifusate by radioimmunoassay (Incstart Corp. kit) using rate insulin (Novo) as standard. To another aliquot of perifusate was added 10 ml scintillation liquid to measure ^{45}Ca efflux from pancreatic islets.

Calmodulin assay. Calmodulin content in pancreatic islets was determined by radioimmunoassay (Amersham kit) in homogenates of 100 pancreatic islets.

DNA assay. DNA was quantified as was described previously[11].

Statistical method. The Newman-Keuls test was applied after analysis of variance.

Results

Basal insulin release in response to 2.7 mM glucose was similar in pancreatic islets of 2-month-old (control), 12-month-old (adult) and 24-month-old (aging) rats.

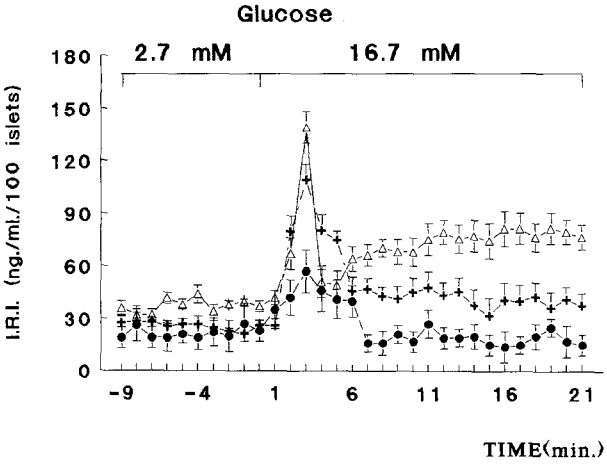

Figure 1. Kinetics of glucose-induced insulin release in young (n = 8) (–△–), adult (n = 6) (–+–) and old (n = 8) (—●—) rats. Results are expressed as means ±SEM ($p < 0.01$).

At the higher doses (16.7 mM), glucose induced a typical biphasic increase in insulin release from islets of young rats. In contrast, the kinetics of insulin release in response to glucose stimulation was altered in adult and aging rats, showing a marked reduction of the second phase of secretion, most noticeably in aging rats (figs 1 and 2).

Decreased glucose uptake in pancreatic islet cells was observed in 24-month-old rats compared with young or adult rats after 2 min of exposure to 16.7 mM glucose.

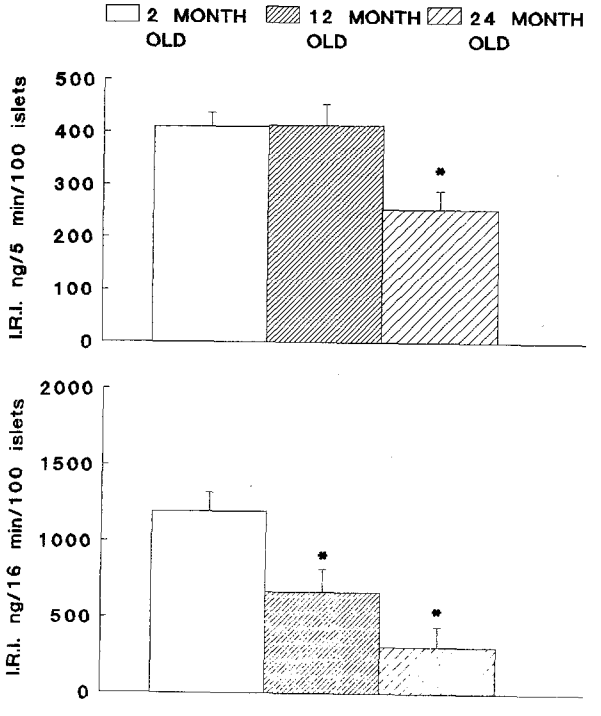

Figure 2. Total values of insulin release during the first (top) and second phases (bottom) in young (n = 6), adult (n = 6) and aging (n = 8) rats. Results are expressed as means ±SEM *($p < 0.01$).

Metabolic studies on pancreatic islets from 2-, 12- and 24-month-old rats

	2-month-old	12-month-old	24-month-old
Glucose uptake mM/10 isl/2 min	6.7 ± 0.3 (n = 8)	6.3 ± 0.2 (n = 6)	2.5 ± 0.17 (n = 8)**
O_2 consumption (%)	100%	96.7%	84.63%*
^{45}Ca uptake µg/10 isl/5 min	1.9 ± 0.23	0.93 ± 0.17	0.7 ± 0.12*
Calmodulin ng/100 isl	6.5 ± 1.51	5.2 ± 1.84	2.17 ± 1.7*

All results are expressed as mean ±SD; *$p < 0.01$. **$p < 0.001$.

In addition, in 24-month-old rats, glucose-induced O_2 consumption was significantly reduced compared to the other two animal groups (table). After exposure for 5 min to 16.7 mM glucose, both ^{45}Ca uptake and calmodulin content were significantly lower in 24-month-old rats (table).

In all groups, 16.7 mM glucose provoked a dual modification of ^{45}Ca efflux: an initial fall was rapidly followed by a secondary rise. No qualitative differences were observed between 2-month, 12-month or 24-month-old rats (fig. 3).

Discussion

We used the isolated, perifused rat pancreatic islet model to study the effect of age on insulin secretory capacity. Basal insulin release in response to the lower concentration of glucose (2.7 mM) was similar in islets from 2-month to 24-month-old rats. This concurs with the results of other authors[12]. Acute elevation of glucose concentration resulted in a typical biphasic insulin secretion pattern in the younger group (2-month-old). However, as we reported recently, dieting and non-dieting elderly rats showed an early spike and decrease

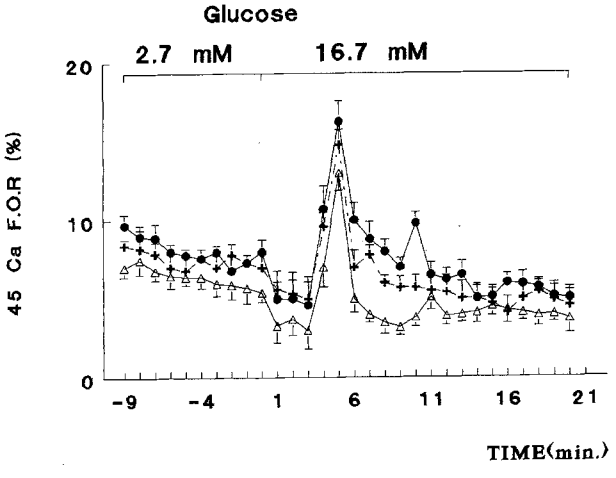

Figure 3. Kinetics of glucose-induced ^{45}Ca fractional outflow rate (%) in young (n = 8) (-△-), adult (n = 6) (-+-) and aging (n = 8) (—●—) rats. Results are expressed as means ±SEM.

and no significant second phase of glucose-induced insulin secretion[2]. Glucose-stimulated insulin release was blunted, as shown by other studies that used different experimental systems[12–14]. Previous results showed a significant attenuation of first phase of insulin secretion[15,16]; the presence or absence of a second phase of insulin release may be a feature of the experimental model. The use of isolated pancreatic islets (either in static incubation or in a perifusion system) may not fully reflect the effect of age on total pancreatic insulin secretion during the aging process, since secretion from isolated islets does not take into account the larger total islet volume of elderly rats compared with young rats. However, this variable was controlled in this study, since pancreatic islet DNA content was similar in all animal groups (data not shown).

The decrease in insulin release by pancreatic islets of older rats has been associated with impaired glucose catabolism[17] and adenylate cyclase activity[12]. Therefore, in our attempts to determine the causes of diminished insulin release in aging, we investigated some indicators of metabolism in the pancreatic islets.

Our findings shed light on the impairment of glucose-stimulated insulin secretion. In our experimental system, the aging process impaired glucose-induced insulin release, with a marked reduction in the second phase of insulin release (fig. 1). This finding is compatible with previous evidence that glucose-induced insulin secretion by isolated pancreatic islets of old rats was 50% lower than in young rats[13]. This decrease is likely to be a consequence of aging, since young (2-month-old) and adult (12-month-old) rats show a similar rate of insulin secretion in response to glucose[2].

Insulin release is directly dependent on the metabolic state of the β-cell[18], so the diminished glucose uptake and O_2 consumption in isolated pancreatic islets from aging rats (table) may be involved in impairment of insulin release. Changes observed in O_2 consumption during aging may be due to the aging process itself, or to decreased glucose uptake in old rats.

The decline in ^{45}Ca and calmodulin content in old rats may be responsible for the impaired ability of pancreatic islets of aging rats to move secretory vesicles to the plasma membrane, as was described previously[19].

In conclusion, several factors may be responsible for the decreased secretory response to glucose stimulation of the pancreatic β-cell in aged rats. Such factors include changes in the metabolic activity of the pancreatic β-cell, together with decreased ^{45}Ca movement and calmodulin content.

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1 Osuna, J. I., Rubio, R., Rodriguez, E., and Osorio, C., in: Biophysics of Pancreatic B Cell, p. 427. Eds I. Atwater, E. Rojas and B. Soria. Plenum Press, New York 1986.

2 Castro, M., Pedrosa, D., and Osuna, J. I., Experientia *48* (1992) 996.
3 Reaven, E. P., Gold, G., and Reaven, G. M., J. clin. Invest. *64* (1979) 591.
4 Molina, J. M., Prendas, F. H., and Lipson, L. G., Endocrinology *116* (1985) 821.
5 Curry, D., Reaven, G. N., and Reaven, E. P., Am. J. Physiol. *248* (1985) E375.
6 Verspohl, E. J., Handel, M., and Ammon, H. P. T., Endocrinology *105* (1979) 1269.
7 Ammon, H. P. T., Grimm, A., Lutz, S., Wagner-Teschner, D., Handel, M., and Hagenloh, I., Diabetes *29* (1980) 830.
8 Ammon, H. P. T., Amm, V., Eusen, R., Hoppe, E., Trier, G., and Verspohl, E. J., Life Sci. *34* (1984) 247.
9 Azhar, S, Ho, N. Y. M., Reaven, E. P., and Reaven, G. M., Horm. Metabol. Res. *20* (1988) 559.
10 Lacy, P. E., and Kostianowsky, M., Diabetes *16* (1967) 35.
11 Eizirik, D. L., Bendtzen, K., and Sandler, S., Endocrinology *128* (1991) 1611.
12 Lipson, L. G., Borrycki, V. A., Bush, M. J., Tietjen, G. E., and Yoon, A., Endocrinology *108* (1981) 620.
13 Gold, G., Reaven, G. M., and Reaven, E. P., Diabetes *30* (1981) 77.
14 Reaven, E. P., Gold, G., Moore, J., and Reaven, G. M., J. clin. Invest. *71* (1983) 345.
15 Denis, D. E., Dana, C. M., Andersen, R., Tobin, J. D., and Andres, R., Endocrinology *118* (1985) 11.
16 Reaven, G. M., and Reaven, E. P., Molec. cell. Biochem. *31* (1980) 37.
17 Reaven, G. M., and Reaven, E. P., Diabetologia *18* (1980) 69.
18 Malaisse, W. J., Herchuelz, A., and Sener, A., in: The Islets of Langerhans: Biochemistry, Physiology and Pathology, p. 149. Eds S. J. Cooperstein and D. Walkin. Academic Press, New York 1981.
19 Draznin, B., Scelinberg, J. P., Leither, W., and Sussman, M. E., Diabetes *34* (1985) 1168.