Impaired Glucose Priming of Insulin Secretion from Perfused Pancreas in Aged Female Rats (43479)

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Abstract. **Effects of age and glucose levels on insulin secretion and synthesis were studied in the perfused pancreas of young (2-month-old) and older (10-month-old) female Wistar rats. Insulin secretion induced by 16.7 m*M* glucose showed a triphasic pattern: an early spike and fall (first phase, 0–6 min), followed by a sustained gradual increase (second phase, 7–120 min) and a gradual decreased release thereafter (third phase, 121–360 min) during the perfusion period of 360 min. First and second phase insulin secretion, but not third phase, were lower in older rats than in young rats. Insulin synthesis in old rat pancreas perfused with 16.7 n*M* glucose for 360 min was much greater than that of young rats. Second phase insulin secretion was restored to comparable levels by 28 m*M* glucose in older rats. Repeated pulses of 28 m*M* glucose potentiated subsequent insulin secretion in young rats, but not in older rats. These findings provide further evidence that sensitivity to glucose in pancreatic B cells is altered by aging.** [P.S.E.B.M. 1992, Vol 201]

Multiphasic insulin secretion from the pancreas occurs in response to a sudden increase in glucose (1–3). Rapid, but transient, release of insulin (first phase) is followed by a progressively increasing release (second phase) over the 60–120 min. A third phase of insulin secretion is characterized by a spontaneous decline during sustained exposure to glucose for up to 48 hr (2–3).

Reaven *et al.* (4) reported that glucose-induced insulin secretory characteristics were not affected as a function of aging. However, it has been reported that total glucose-stimulated insulin secretion was unchanged (4–6), suppressed (7), or increased (8) in aged rats. These variable observations may be due to the experimental models used and the levels of glucose stimulation (5). Although male rats were used in most reports (4–8), their body weights increase considerably with age, and endocrine function may be affected by obesity itself. There are few studies on the effect of aging on insulin secretion from the pancreas of female rats, in which body weight gain with age is less prominent than in male rats (9).

Curry and MacLachlan (10) demonstrated that long-term (360 min) stimulation with near-maximal glucose (16.7 m*M*) resulted in an age-related increase in rat insulin synthesis, which was defined as *de novo* synthesis and conversion of existing preproinsulin and proinsulin to insulin and less intracellular degradation of insulin (11).

Repeated administration of a stimulating level of glucose potentiates the insulin response to subsequent stimulation (priming effect) in humans (12) and rats (13, 14). Although the exact mechanism remains to be fully elucidated, the priming effect of glucose was not demonstrated in the mouse (15) and diabetic rats (16). It remains unclear whether glucose priming is preserved in aged pancreatic B cells.

In the present study, we examined the effect of glucose stimulation for a short and long period on insulin secretion in perfused rat pancreas and the priming effect of glucose in aged female rats.

Materials and Methods

Animals and Perfusion of the Pancreas. Female Wistar rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were purchased at 6 weeks of age. The

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animals were housed in air-conditioned quarters at 24°C under artificial lighting (lights on 0800–2000 hr). Tap water and laboratory chow pellets (Japan Crea Co., Tokyo, Japan) were given *ad libitum*. The animals were divided into two groups and used for experiments at the age of 2 months and 10 months, respectively. All experiments were conducted on nonfasted animals and started between 0900–1200 AM.

Blood samples (0.2 ml) were collected from the tail vein without anesthesia. Then, the animals were anesthetized with pentobarbital (35 mg/kg, ip) and the pancreas was isolated as described by Goto *et al.* (17). The isolated pancreas was perfused with Krebs-Ringer bicarbonate buffer containing 4.4 m*M* glucose, 0.25% bovine serum albumin, and 4.8% dextran (mol wt 70,000), as reported previously (17, 18). The Krebs-Ringer bicarbonate buffer was gassed with 95% O_2 and 5% CO_2 and maintained at pH 7.4 and 37°C. The flow rate was kept constant at 1.9 ml/min by the use of a perifusion pump (Nihon Rika, Tokyo, Japan). After an equilibration period of 20 min, the glucose concentration was raised to 16.7 m*M* through a side pump without changing the flow rate. After the pancreas was perfused with 16.7 m*M* glucose for 20 min, the glucose level was lowered to the initial concentration of 4.4 m*M* and perfused further for 20 min. The perfusate was collected every 1 min for 60 min in sampling tubes, frozen immediately, and stored at −20°C until assayed.

In the second series of experiments, 16.7 m*M* glucose were perfused for a longer period of 180 or 360 min after an equilibration period, and the perfusate was collected at an interval of 4–20 min during the perfusion.

In the third series of experiments, the priming effect of glucose was studied according to the method described by Grill and Rundfeldt (16). The pancreas was initially perfused with 4.4 m*M* glucose for 20 min, subsequently with 28 m*M* glucose for 20 min, and then with 4.4 m*M* glucose for 20 min again, which was followed by the perfusion with 28 m*M* glucose for 20 min. The perfusate was collected every 1 min during the period of the perfusion.

Extraction of Insulin. The pancreas was removed immediately after termination of the perfusion, minced, sonicated in 20 vol of cold acid-ethanol (0.18 *M* HCl in 75% v/v ethanol), and extracted for 16 to 18 hr at 4°C. After centrifugation at 2000*g* for 30 min at 4°C, aliquots of the supernatant were stored at −70°C until assayed. The pancreas without the perfusion was also extracted as a control.

Assays. Immunoreactive insulin levels in the plasma, perfusates, and extracts were measured by specific radioimmunoassay, as described previously (18), using rat insulin (Novo, Bagsvaerde, Denmark) as a standard. The minimum detectable insulin level was 6 μU/ml in the assay, and the interassay and intra-assay variations were 6% and 9%, respectively. Insulin levels in the extract were corrected by the recovery rate (70–80%) of the standard insulin extracted by the same method. Plasma glucose was measured by the glucose oxidase method.

Calculation of Insulinogenesis. Insulinogenesis was calculated by the procedure of Curry (11) as

$$I_G = I_R + I_{Ce} - I_{Cb}$$

In which I_G is insulinogenesis and I_R is total insulin released by a perfused pancreas during the perfusion period and estimated from insulin concentrations in the perfusate multiplied by the flow rate and the perfusion period. I_{Ce} and I_{Cb} are insulin content in a pancreas at the end of the perfusion and without the perfusion, respectively. I_{Cb} was determined in 10 control animals of each age group.

Statistical Evaluation. Statistical analysis was performed by unpaired Student's *t* test when two mean values were compared and Scheffe's method when three mean values were used.

Results

The body weights were greater in older rats than in young rats (mean ± SE, 316.3 ± 3.1 g vs 190.0 ± 2.5 g, $P < 0.005$). Wet weights of the pancreas were not different between young and older rats (2.06 ± 0.13 g vs 2.49 ± 0.19 g). Plasma glucose and insulin levels were in the normal range in both animal groups (glucose, 8.1 ± 0.2 m*M* vs 8.6 ± 0.2 m*M*; insulin, 79.2 ± 2.7 μU/ml vs 93.5 ± 7.0 μU/ml).

Biphasic insulin secretion was obtained in both young and older rats when the pancreas was perfused with 16.7 m*M* glucose for 20 min (Fig. 1). The peak value of insulin concentration in the first phase (at 4 min) was much greater in young rats than in older rats

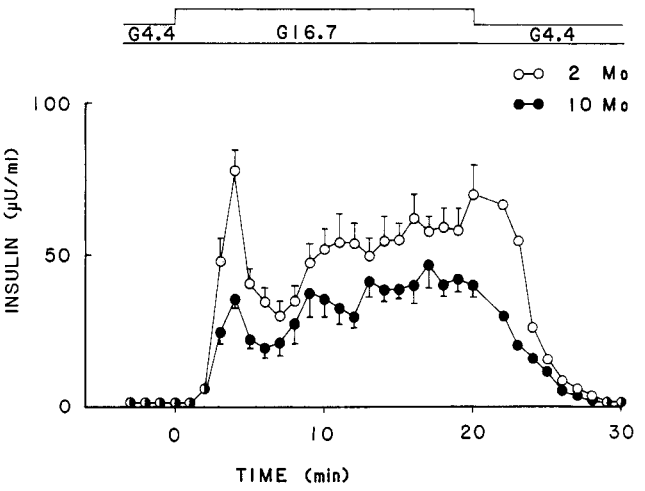

Figure 1. Insulin release (mean ± SE) from the isolated pancreas perfused with 16.7 m*M* glucose for 20 min in 2-month-old and 10-month-old female rats.

(79.9 ± 6.8 μU/ml vs 35.6 ± 1.6 μU/ml, $P < 0.005$). The amount of cumulative insulin release in the second phase (7–20 min) was also blunted in older rats (1.42 ± 0.17 mU/pancreas vs 0.97 ± 0.06 mU/pancreas, $P < 0.05$).

When the pancreas was perfused with 16.7 m*M* glucose for 180 min, insulin release initially peaked at 4 min, then gradually increased to a second peak at 120 min and decreased toward 180 min in both young and older rats (Fig. 2). The amount of total insulin release during the perfusion period of 180 min was significantly lower in aged rats than in young rats (67.1 ± 6.8 mU/pancreas vs 95.6 ± 11.4 mU/pancreas, $P < 0.05$).

When the pancreas was perfused with 16.7 m*M* glucose for 360 min, insulin release was considerably decreased from 121 to 360 min after the start of 16.7 m*M* glucose (third phase) in both animal groups (Fig. 3). The amount of insulin released in the first and second phases (0–120 min) was much greater in young rats than in older rats (65.9 ± 9.3 mU/pancreas vs 20.9 ± 1.2 mU/pancreas, $P < 0.05$). However, the cumulative insulin release in the third phase was slightly, but not significantly, increased in older rats (150.8 ± 17.2 mU/pancreas vs 85.4 ± 5.5 mU/pancreas).

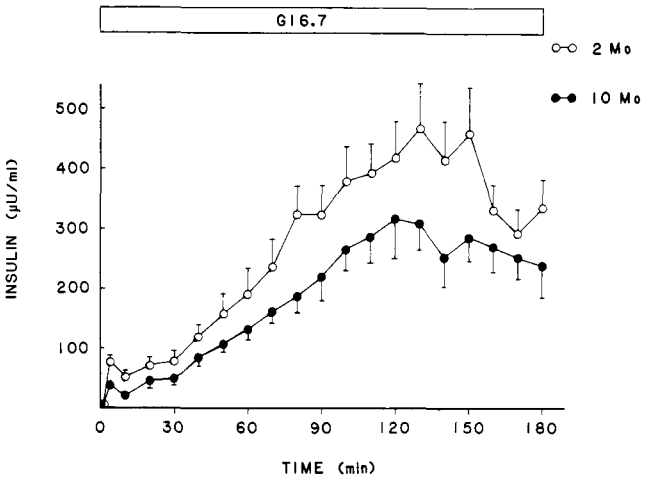

Figure 2. Insulin release (mean ± SE) from the isolated pancreas perfused with 16.7 m*M* glucose for 180 min in 2-month-old and 10-month-old female rats.

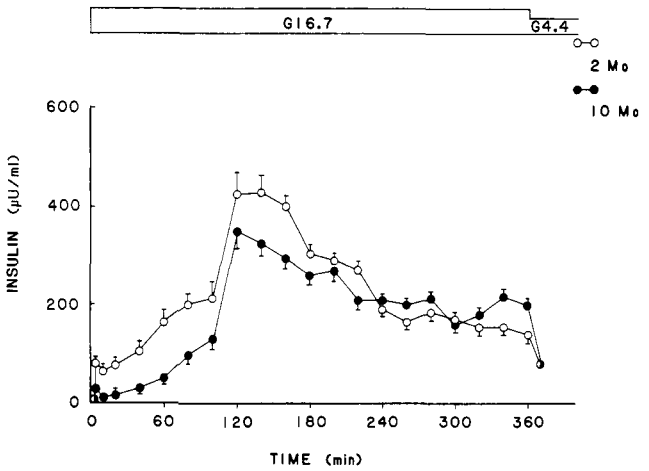

Figure 3. Insulin release (mean ± SE) from the isolated pancreas perfused with 16.7 m*M* glucose for 360 min in 2-month-old and 10-month-old female rats.

The insulin content in the pancreas without the perfusion was greater in older rats than in young rats (1206.5 ± 39.0 mU/pancreas vs 843.9 ± 37.2 mU/pancreas, $P < 0.05$) (Table I). Insulinogenesis occurred in older rats when the pancreas was perfused with 16.7 m*M* glucose for 180 min. When glucose stimulation was prolonged up to 360 min, insulinogenesis was 2-fold greater in older rats than in young rats (817.9 ± 52.6 mU/pancreas vs 414.0 ± 37.6 mU/pancreas, $P < 0.05$) (Table I).

Insulin secretion was remarkably stimulated by 28 m*M* glucose that was perfused for the initial 20 min and was blunted during the next 20-min period in which 4.4 m*M* glucose was perfused (Fig. 4). There was a slight but significant difference in the amount of insulin released in the first phase (0–6 min) between young and older rats (2.5 ± 0.5 mU/pancreas vs 1.6 ± 0.1 mU/pancreas, $P < 0.05$). The amount of insulin release in the second phase (7–20 min) was not different between the two age groups (8.2 ± 1.0 mU/pancreas vs 8.7 ± 0.1 mU/pancreas). When the pancreas was further perfused with 28 m*M* glucose as the second stimulant, the amount of insulin released in the second phase was much greater in young rats than in older rats (12.6 ± 1.9 mU/pancreas vs 8.6 ± 0.4 mU/pancreas, $P < 0.05$). The peak insulin level in the first phase was slightly, but not significantly, enhanced by the second stimulation with 28 m*M* glucose in older rats (512.7 ± 7.0 μU/ml vs 376.0 ± 58.3 μU/ml).

Discussion

Perfusion of female Wistar rat pancreas with 16.7 m*M* glucose for either 20 or 180 min caused biphasic release of insulin in both age groups of rats. Insulin release in the first phase, as well as in the second phase, was lower in aged rats than in young rats. However, when the pancreas was perfused with 28 m*M* glucose, the amount of cumulative insulin release in the second phase in older rats was not different from that in young rats. The diminishing age effect with increasing glucose dose was reported previously in male Wistar rats (5). These findings indicate that impaired insulin response to glucose in aged rats is not due to a diminished capacity to secrete insulin, but due to a defect in sensitivity to glucose.

Curry (11) reported that total insulin secretion, as well as insulinogenesis, in the third phase (2–6 hr) was elevated in older male Sprague-Dawley rats. In the present study, we found that insulinogenesis was much greater in older female Wistar rats than in young rats,

Table I. Effect of Physiological Glucose (16.7 m*M*) on Insulin Release, Insulin Content, and Insulinogenesis in Perfused Pancreas of 2-Month- and 10-Month-Old Female Rats

	Perfusion period (min)	*n*	Insulin (mU/pancreas)		
			Release	Content	Insulinogenesis
2-month-old rats	0	10	ND	843.9 ± 37.2	ND
	180	5	95.6 ± 11.4	782.7 ± 91.0	ND
	360	3	152.8 ± 32.5	1108.2 ± 14.2	414.0 ± 37.6
10-month-old rats	0	10	ND	1206.5 ± 39.0[b]	ND
	180	5	67.1 ± 6.8[b]	1274.0 ± 162.0[b]	149.3 ± 21.0[b]
	360	3	179.4 ± 17.3	1845.0 ± 45.8[b]	817.9 ± 52.6[b]

[a] Means ± SE values are shown. ND, not detectable.
[b] $P < 0.05$, compared with 2-month-old rats.

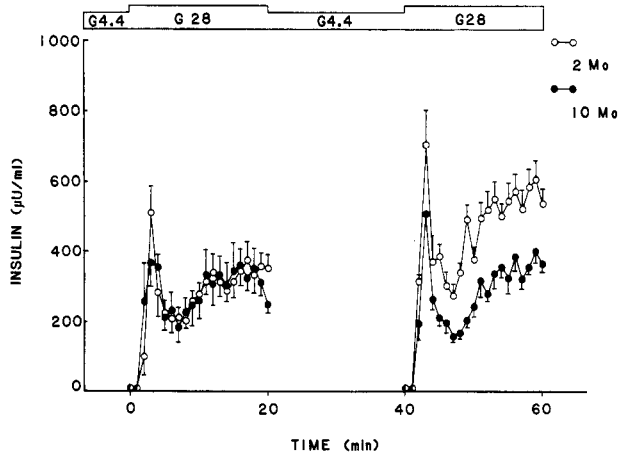

Figure 4. Insulin release (mean ± SE) from the isolated perfused pancreas of 2-month-old and 10-month-old female rats. Repeated 20-min pulses of 28 m*M* glucose with 20-min rest periods of 4.4 m*M* glucose were given.

whereas third phase (121–360 min) insulin secretion was slightly, but not significantly, greater in older rats than in young rats. Insulin synthesis rates were greater in older rats than in young rats (11.3% vs 8.2%/hr), although these values are substantially less than the very high levels reported by Curry (10) and Curry and MacLachlan (11). Wang *et al.* (19) reported that preproinsulin mRNA were not changed, but proinsulin synthesis and the secretion of newly made insulin were decreased in pancreatic islets of old male Fischer rats. These findings suggest that glucose-induced secretion of newly made insulin may be impaired, whereas insulin biosynthesis is maintained in aged female Wistar rats.

Repeated pulses of 28 m*M* glucose did not potentiate subsequent insulin release in older rats. The lack of a priming effect of glucose was reported previously in the mouse, in which the second phase of insulin secretion was markedly blunted (15). Berglud (15) attributed the lack of glucose priming to the absence of second phase insulin secretion.

Insulin release induced by glucose is mediated by intracellular second messengers such as cAMP, C kinase, and Ca^{2+}. We reported previously that impaired second phase insulin release in older rats was potentiated by 12-O-tetradecanoylphorbol 13-acetate, an activator of C kinase, and also by 3-isobutyl-1-methylxanthine, a stimulant of cAMP production (18). It was demonstrated that the biphasic changes in calcium concentrations in the cytosol pool were closely related to the biphasic pattern of glucose-stimulated insulin release (20). It was also reported that glucose oxidation was impaired in aged rat islets (21). Therefore, the lack of glucose priming of insulin secretion may be explained by altered intracellular mechanisms after recognition of glucose.

The lack of glucose priming was also reported previously in neonatally streptozocin-induced diabetic rats and in dexamethasone-treated rats (16). When streptozocin-induced diabetic rats were fasted or treated by insulin for 36 hr, induction of glucose priming reappeared, which suggests that chronic hyperglycemia leads to a loss of the induction of glucose priming. Dexamethasone-treated rats did not display overt hyperglycemia, which indicates that increasing demand on B cell function may exhaust the capacity to induce priming before overt hyperglycemia ensues (16). However, hyperglycemia and hyperinsulinemia were not shown in older female rats in the present study.

It is concluded that first and second phase insulin secretion and glucose priming are impaired in aged female rats.

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