Effect of Age and Sex on Rat Endocrine Pancreas

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SUMMARY

Maximal glucose-stimulated insulin secretion was quantified in perfused pancreases of 11-wk-old and 12-mo-old female and male rats. In addition, measurements were made of body weight, total pancreatic weight, and percentage of the pancreas occupied by islet tissue. Body weight (mean ± SE) of male rats was greater than that of female rats at both 11 wk (319 ± 3 vs. 237 ± 13 g) and 12 mo (684 ± 17 vs. 376 ± 13 g) of age. Pancreatic weight and percentage of the pancreas occupied by islet tissue were also greater in male rats and increased in approximate proportion to the gain in weight. The first phase and the second phase of maximal glucose-stimulated insulin secretion were both qualitatively and quantitatively similar in all four groups of rats. However, because islet cell mass increased with age, maximal glucose-stimulated insulin secretion declined with age in rats of both sexes when expressed per unit islet tissue. Although the fall in insulin secretion (per islet cell mass) with age was observed in perfused pancreases from both male and female rats, the pancreases of female rats contained relatively less islet tissue and secreted more insulin per unit islet cell mass than pancreases of male rats at either age. Thus, there are sex differences in both islet cell structure and function, but the effect of age on endocrine pancreatic function seems to be independent of sex.

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Previous studies from our laboratories have demonstrated that maximal glucose-stimulated insulin secretion per β-cell declines progressively as male rats age (1–5). These studies have been carried out predominantly in Sprague-Dawley rats that become obese as they grow older, and we have attempted to control for the effects of obesity by caloric manipulation. With this approach we have shown that the decline in maximal glucose-stimulated insulin secretion per β-cell that occurs with age is not alleviated when obesity is prevented by feeding rats calorie-restricted diets as they grow from 2 to 12 mo of age (3–5). On the other hand, it could be argued that feeding rats a calorie-restricted diet for 10 mo may itself adversely affect insulin secretory function. To address this issue, we decided to study untreated female Sprague-Dawley rats, who normally gain less weight with age than their male counterparts. As a result, the weight difference between young and old female rats is much less than that between young and old male rats. In this fashion we compared maximal glucose-induced insulin secretion in young and old rats of relatively similar weights without the confounding variable of calorie restriction. These studies would also permit us to see if the age-related defect in insulin secretory function was sex specific. The results provide further evidence for the view that maximal glucose-induced insulin secretion per β-cell declines with age, and that this change appears unrelated to obesity. Because the age-associated decline in insulin secretory function was seen in both male and female rats, this change does not appear to vary as a function of sex. On the other hand, significant differences in both the structure and the function of the endocrine pancreas from male and female rats were noted.

MATERIALS AND METHODS

Female and male Sprague-Dawley rats were obtained from Bantin & Kingman (Fremont, CA) at 10 wk of age and maintained in our facilities for aging animals until used for study at 11 wk or 12 mo of age. The rats were bedded on Beta Chips (Northeastern, Warrensburg, NY), housed two rats per cage (19 × 10.5 × 8 inches) in laminar flow racks (Lab

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Products, Rochelle Park, NJ), and fed standard Purina lab chow (no. 5012; Ralston Purina, Chicago, IL) ad libitum.

Isolated pancreases from 5–8 rats of each of the four groups were used for perfusion studies to determine maximal glucose-stimulated insulin secretion. Perfusion experiments in this study were carried out during a 1-wk period and were initiated between 1000 and 1400 h; an attempt was made to mix rats of different sex and/or age in each day's perfusion schedule. Perfusion techniques were as previously described (5). The media contained Krebs-Ringer bicarbonate buffer, 4% dextran, 0.2% bovine serum albumin, and glucose (either 2.8 mM glucose for the 10-min equilibration and 2-min prestimulation period or 16 mM glucose for the 58-min stimulation interval). The choice of 16 mM glucose as a maximal stimulant for the four groups of rats was based on information obtained from an earlier study (1). Perfusate temperature was 37°C, and flow rate was kept constant at 10 ml/min. At the end of the perfusion period, formalin (10% phosphate buffered) was infused through each pancreas, and the tissue was stored in fixative pending morphometric evaluation. Perfusate samples were assayed for insulin by radioimmunoassay (6).

To obtain pancreas weights, pancreases of five additional rats of each category were inflated with distilled water, excised, cleaned of adherent fat and connective tissue with the aid of a dissecting microscope, lyophilized, and weighed (5). These rats were obtained from the same vendor, arrived the same day, and were maintained in our facilities for aging animals under the same conditions as the rats used for perfusion studies.

Paraffin sections of the splenic region of each perfused pancreas used in the study were analyzed morphometrically to determine the percentage of islet tissue per unit pancreas tissue as previously described (5), as well as to determine average islet size. Small differences between numbers in this study and previous studies may be accounted for by the fact that we are assessing the volume of tissue compartments (e.g., ratio of endocrine to exocrine tissue) with a computerized image-analysis system (Bioquant II; r & m Biometrics, Nashville, TN), whereas previous measurements were carried out with the grid method for point-counting stereology (7). In brief, planimetric measurements were made on one hematoxylin-eosin–stained section (~15 × 15 mm) of one block from the splenic portion of each perfused pancreas. Low-magnification images (×107) of all portions of each section of pancreas were displayed on the video screen, and sequential additive measurements were made of the total section area, including the islet tissue. Exocrine interlobular space visible at this magnification was excluded from the planimetric measurements. Area measurements were automatically converted to volume measurements by the computerized program. For measurements of islet tissue, the entire block face was reexamined at somewhat higher magnification (×266) and each islet profile of the 30–60 islets/section was separately measured. Under the experimental conditions used, islet profiles consisting of only 3–4 cells were clearly identifiable and were included in the measurements. Prominent bands of islet connective tissue (as seen in islets of aging male rats) were also visible and were excluded from measurements. Total islet volume divided by total section volume provided an estimate of the percentage of islet tissue per unit pancreas tissue. An estimate of islet diameter was also obtained from the individual islet volume measurements of each pancreas. As previously described, pancreatic insulin secretion rates [with the average for second-phase secretion (30–60 min) of each animal] were subsequently corrected for total pancreas weight and percentage of islet tissue per unit pancreas and expressed as insulin secretion per time per islet tissue mass (5). Differences between groups were assessed by Student's nonpaired *t* test.

TABLE 1
Effect of aging on body weight of male and female Sprague-Dawley rats

	n	Body weight (g)
Females		
11 wk old	5	237 ± 5
12 mo old	8	376 ± 13
Males		
11 wk old	5	319 ± 3
12 mo old	8	684 ± 17

Data are expressed as means ± SE. Each group has significant difference of $P < .01$ vs. other 3 groups.

RESULTS

The effect of age on the body weight of the four groups of experimental animals is given in Table 1. It is apparent from the data that 11-wk-old female rats were ~80 g lighter than

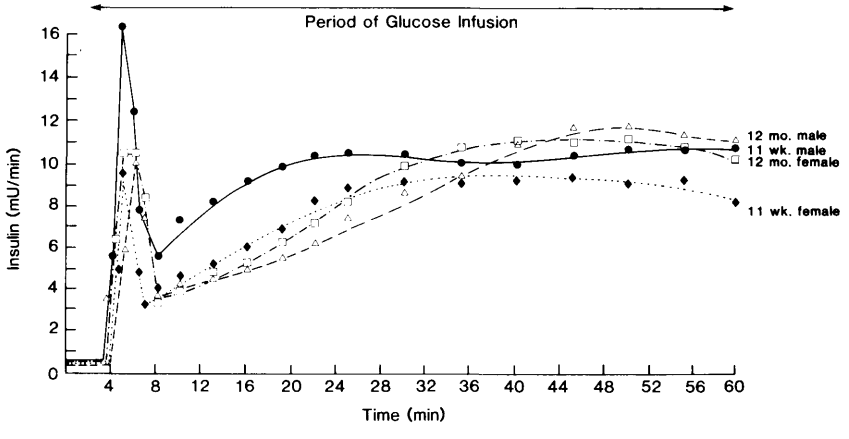

FIG. 1. Time course of maximal (16 mM) pancreatic glucose-stimulated insulin release by perfused pancreases of 11-wk-old (*n* = 5) and 12-mo-old (*n* = 8) male and female rats. Insulin (mU/min) secretion is biphasic, with first-phase response at 4–8 min and second-phase response that reaches steady-state values at 30–60 min.

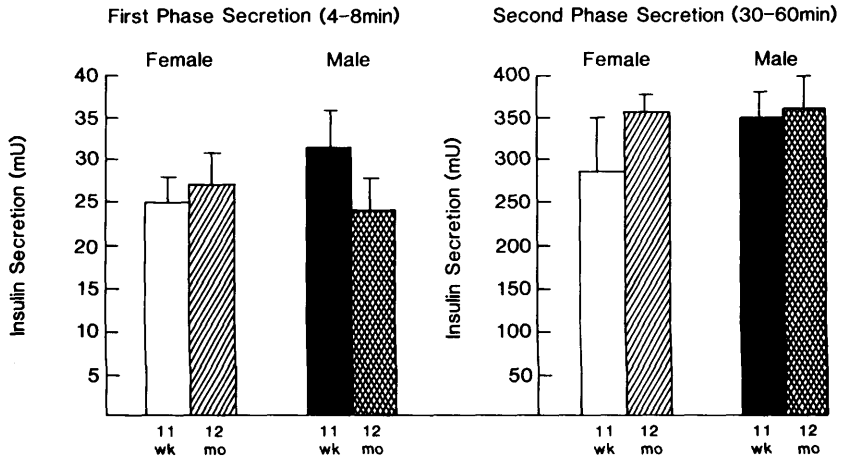

FIG. 2. Total insulin (mean ± SE) released by perfused pancreases of 4 groups of rats during first (4–8 min) and second (30–60 min) phases of insulin response.

11-wk-old male rats, whereas the difference in weight between rats of the two sexes averaged ~300 g at 12 mo of age. These figures demonstrate that the rate of weight gain with age is less in female rats.

Mean insulin secretory responses to 16 mM glucose are seen in Fig. 1. These curves show that total insulin release (mU/min) over time in all four groups of rats follows the usual biphasic response patterns. Furthermore, total insulin secretory response to glucose is approximately the same in all four groups.

For comparison, we calculated the total amount of insulin released during the first phase (4–8 min) and during the steady-state portion of the second phase of insulin secretion (30–60 min). These data are shown in Fig. 2 and again document the similarity in the insulin secretory response of the four groups of rats studied.

The effect of age and sex on both the total weight of the pancreas and the percentage of the pancreas occupied by endocrine tissue is seen in Figs. 3 and 4. It is apparent from Fig. 3 that at each age the pancreas weighed less in females, and the incremental increase in pancreatic mass with age was less in the female rats. In general, the increase in weight of the pancreas with age tended to parallel the changes in body weight seen in Table 1. In addition, aging was associated with an increase in islet size; i.e., estimates of mean ± SE islet diameter rose from 172 ± 18 μm (11 wk) to 250 ± 24 μm (12 mo) in female rats ($P < .01$) and from 192 ± 18 μm to 272 ± 18 μm ($P < .01$) in male rats. When corrected to volume, these measurements suggest that there was an approximate doubling of islet volume as rats of both sexes grew from 11 wk to 12 mo of age. It is important to note that regions of the islets containing connective tissue [found especially in islets of aging male rats (3,5)] were not included in the measurements. The increase in islet size with age was reflected in a significant increase in the percentage of the total pancreas that was occupied by islet tissue as the rats aged from 11 wk to 12 mo. The magnitude of the changes noted are seen in Fig. 4, and it is apparent that this finding was true of both female and male rats.

Based on the values for total pancreatic mass and percentage of the pancreas occupied by islet tissue shown in Figs. 3 and 4, it was possible to express the insulin secretory period data as a function of islet cell mass. Figure 5 shows this for the insulin released at 30–60 min (steady state, second phase). It appears from these data that insulin secretion (corrected for islet cell mass) declined significantly with age ($P < .001$) in both sexes. However, when compared at comparable ages, the insulin response remained significantly greater ($P < .001$) in female rats than in male rats.

DISCUSSION

At the simplest level, these results have again shown that maximal glucose-stimulated insulin release per unit islet cell mass declines as rats grow older. This change was observed in both female and male rats and appears to be independent of degree of obesity. That is, aging female rats gained less than half the body weight gained by the male rats, but, like

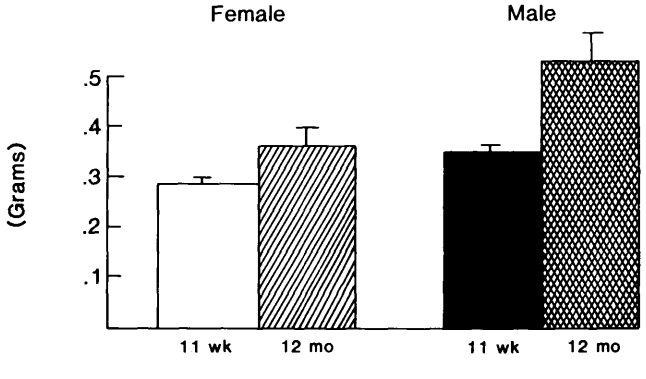

FIG. 3. Pancreas dry weights from 11-wk-old and 12-mo-old male and female rats comparable in body weight to rats used for perfusion studies. Data are means ± SE of pancreases of 5 rats in each category.

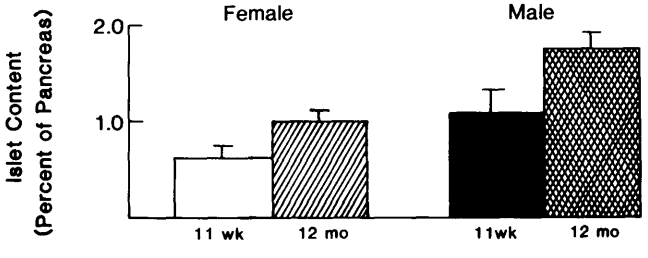

FIG. 4. Islet content per unit volume of pancreatic tissue. Data are means ± SE from formalin-fixed pancreases of each animal used for perfusion studies in Fig. 1.

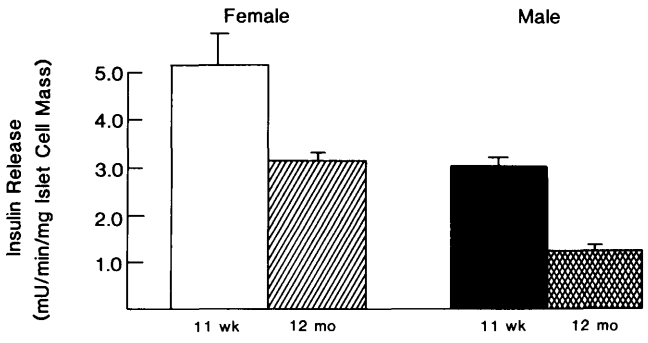

FIG. 5. Second-phase insulin release (30–60 min) by perfused pancreases of 11-wk-old and 12-mo-old male and female rats (Figs. 1 and 2) corrected for pancreas size (Fig. 3) and islet content (Fig. 4). Calculations were made with following formula: insulin release per milligram islet cell mass (mU/min) = [second-phase insulin secretion per pancreas (mU/min)]/[pancreas dry wt (g) × islet content of pancreas (%) × 10].

the male rats, the older females had substantially higher values for endocrine cell mass than young rats, and insulin secretion corrected for this endocrine mass was relatively reduced. As a result, insulin secretion rates declined in older rats of both sexes. Although it is theoretically possible that age-related changes within the islets could have resulted in abnormally few β-cells per unit islet tissue (despite the measured increase in islet mass), this is not a likely explanation for the observed decrease in insulin secretion. For one, all nonendocrine portions of the islets (e.g., connective tissue) were excluded from measurements of islet volume and could not have artifactually increased the values for islet content per pancreas. In addition, we have previously shown that the relative number of β-cells within enlarged islets of aging rats actually increases (at the expense of other endocrine cells such as α-cells), not the other way around (9). As a result, measurements of islet cell mass, as done in this study, probably underestimate the true β-cell mass of the older animals and therefore may underestimate the extent of the age-related decline in the insulin response. Together these data support our previous observations in calorie-restricted male Sprague-Dawley rats that demonstrated an age-associated decline in insulin secretory function independent of obesity (3–5). We have also noted a decline with aging in maximal glucose-stimulated insulin secretion by Fisher rats (8). Thus, loss of insulin secretory function with age appears to be independent of obesity, sex, and species of rat, strongly suggesting that it is a true consequence of the process of aging.

Although the decline in insulin secretory function associated with aging can be documented in rats of either sex, these results clearly indicate that substantial differences in both structure and function of the endocrine pancreas are seen when female rats are compared with male rats. Values for total pancreatic weight and percentage of pancreases occupied by islet tissue are significantly lower in 11-wk-old and 12-mo-old females than in similarly aged male rats. However, total glucose-stimulated insulin secretion by perfused pancreases of male and female rats are comparable. Consequently, insulin secretion per unit islet cell mass is significantly greater in perfused pancreases of females compared with male rats, and this difference is independent of age. Thus, although female rats have a smaller pancreatic pool of β-cells than comparably aged male rats, their β-cells appear to secrete insulin more efficiently. It is interesting that insulin-stimulated glucose uptake by isolated adipocytes from female rats is also more efficient than in adipocytes from male rats (10), and there may be a connection between the enhanced insulin sensitivity of adipocytes from female rats and the fact that their islet cells are also more sensitive to the stimulatory effect of insulin. The reasons for these differences in insulin secretion and action are not explicable by current data but represent important physiological differences that merit additional study.

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