Effect of Age and Environmental Factors on Insulin Release from the Perfused Pancreas of the Rat

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ABSTRACT In this study we examined the effect of age and various age-related environmental factors on maximal glucose-stimulated insulin release by the intact perfused pancreas. Male Sprague-Dawley rats were maintained from 40 d to 12 mo of age on standard chow, or on a sucrose-rich or calorie-restricted diet. At 12 mo, studies were carried out on the isolated pancreas of each animal to determine maximal (300 mg/ml) glucose-stimulated insulin secretion. After these studies were completed, each pancreas was perfused with formalin fixative and processed for morphometric estimation of the mass of the endocrine pancreas. Data from these older animals were compared with data from 2-mo-old control rats. The results indicate that maximal glucose-stimulated insulin secretion per unit endocrine pancreas was markedly reduced in all three groups of 12-mo-old rats, and was only 25–33% of that of 2-mo-old rats. Thus, aging led to a decline in insulin secretion per beta cell that was not modifiable by environmental manipulation. On the other hand, environmental factors can influence the development of endocrine tissue within the pancreas, and in so doing, modify total pancreatic insulin secretion. The mass of the endocrine pancreas of 12-mo-old rats fed either sucrose or chow was between three and four times that of 2-mo-old control rats, and these older rats were able to maximally secrete as much insulin per total pancreas as the young rats. In contrast, the endocrine cell mass of the calorie-restricted rats had not enlarged to this extent, and the maximally stimulated perfused pancreas from these rats secreted less insulin. These data suggest that the aging animal, challenged in vivo to secrete insulin, can overcome the loss of the beta cell response by expanding its pancreatic pool of beta cells. Although this compensation is successful in the 12-mo-old, obese, middle-aged rat, it is not yet clear what effect further aging would have on these events.

INTRODUCTION

Previous studies have shown that collagenase-isolated islets of aging animals show decreased capacity to secrete insulin in response to both glucose (1–4) and leucine (5) stimulation. This deterioration of the islet response can be detected in rats by 6 mo of age, worsens with each 6-mo-interval, and occurs despite the fact that the insulin content of the islet increases with age (1). Calculations that take into account the fact that the number of beta cells increases in the average islet from older rats, indicate that the observed age-related decline in islet secretion is a reflection of a decreased response of the beta cell itself (1).

Although these experiments strongly suggest that insulin secretion deteriorates with age, several unanswered questions remain. First among these is the problem of whether the age-related reduction in insulin secretion, noted in vitro, is an artifact of the process by which the islets are isolated. This concern is not entirely theoretical, as we know that there is often an increase in the connective tissue content of islets from older animals (2), and the possibility exists that islets from aging animals may be more susceptible to the action of collagenase than islets from young animals. The second question concerns the impact that an age-related decline in the secretory capacity of individual beta cells may have on the total secretory response of the animal. Clearly, the insulin secreted by the total pancreas of an animal will depend not only on the insulin released from individual beta cells, but also on the total number of beta cells within the pancreas. Finally, we feel it is important to determine

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whether the beta cell changes that have been observed are an inevitable function of aging (and therefore are not modifiable), or whether they reflect changes induced by certain environmental factors which often accompany aging.

The current study is an attempt to respond to all three of these issues. For this purpose we have used the technique of extracorporeal organ perfusion, and documented the effect of age, obesity, and diet on maximal glucose-stimulated insulin release by the whole rat pancreas. By combining these functional measurements with morphological observations, we have been able to determine the effect of these variables on total pancreatic insulin secretion, insulin secretion per unit pancreas weight, and insulin secretion per unit endocrine pancreas. The results to be presented indicate that age leads to a profound decrease in maximal glucose-stimulated insulin secretion when considered per unit endocrine pancreas, and that this decline in beta cell function is not affected by changes in diet or degree of obesity. On the other hand, variations in the amount or kind of calories consumed by the aging animal can significantly affect the total mass of the endocrine pancreas, and this, in turn, dramatically influences the ability of the whole pancreas to respond to a glucose challenge.

METHODS

Experimental protocol

18 male, Sprague-Dawley rats (caesarean-originated, barrier sustained) were obtained from the Charles River Breeding Laboratories (Wilmington, MA) at 40 d of age. 5 d after arrival the rats were individually tagged with ear clips and weighed. Subsequently, the animals were housed three to a cage, placed in laminar flow hoods (Lab Products, Inc., Rochelle Park, NJ) to decrease the chance of infection, and six rats each were maintained on the following diet programs until they were killed at 12 mo of age.

Chow-fed rats. Rats in this category ate standard laboratory chow (Purina Chow pellets [Ralston Purina Company, Chicago, IL]; 350 kcal/100 g) ad lib. It was anticipated that these rats would be sedentary throughout their lives, and would become relatively obese (2) and insulin resistant as they aged (2, 6).

Sucrose-fed rats. These animals were fed a pellet diet (350 kcal/100 g), rich in sucrose, and containing (as percent total calories) 66% sucrose, 22% casein, and 12% lard; (Tek Labs, Madison, WI). The rats ate this diet ad lib., and based on previous short term studies (7), it was expected that they would gain weight at the same rate, but become significantly more insulin resistant than rats eating standard laboratory chow.

Calorie-restricted rats. Rats in this category were fed a pellet diet (114 kcal/100 g), containing one-third Purina Chow and two-thirds cellulose (Alphacel; Tek Labs). Previous studies had indicated that rats fed this diet ad lib. would gain substantially less weight than the animals in the other categories, and would presumably be less insulin resistant as they aged (2).

At 12 mo of age, studies were carried out on the isolated pancreas of each animal to determine maximal glucose-stimulated insulin secretion as described below. At the conclusion of the secretory study, each pancreas was further perfused with 10% buffered formalin for 10 min, and was subsequently stored in fixative for several days before being processed for morphological studies. For comparison, six young male, Sprague-Dawley rats (~250 g) were directly obtained from the original breeder (Charles River) and studied in an identical fashion.

Secretion studies

Pancreatic perfusions were carried out between 1000 and 1400 h, and perfusion times were varied so that the time of day that a particular experiment was performed did not constitute a systematic variable. These studies were carried out as previously described by Curry et al. (8, 9). In brief, the pancreas was isolated, the superior mesenteric artery ligated, and cannulas were inserted into the celiac artery and portal vein. It should be noted that this procedure effectively removes the ventral-duodenal portion of the pancreas from the perfusion process. Perfusate solutions contained modified Krebs-Ringer bicarbonate buffer (pH 7.4, 30°C), dextran (4%, average mol wt = 73,000), human albumin (0.2%), and various levels of glucose (9). The perfusate was equilibrated in an atmosphere of 5% CO_2 and 95% O_2. Each pancreas was perfused without glucose for a period of 10 min at a flow rate of 5 ml/min to allow for basal state equilibration and temperature stabilization. After this initial period, a maximal stimulating dose of glucose (300 mg/dl) (10) was infused for the following 60 min. During this period of perfusion, sampling was done at 30-s intervals for the first 6 min, at 1-min intervals until 10 min, at 3-min intervals until 25 min, and at 5-min intervals thereafter. No adjustments in flow rate were made for variations in animal size, as preliminary experiments (unpublished observations, D. Curry) showed that in rats weighing from 250 to 450 g, a wide range of perfusion flow rates (3 to 20 ml/min) did not influence insulin secretion rates.

Venous effluent samples were assayed for insulin concentration (microunits of insulin per milliliter), and total insulin secretion during each time period was corrected by multiplying concentration by flow rate. First-phase secretion was taken to be total insulin secreted from 1 to 8 min. Steady-state second-phase insulin secretion was taken to be total insulin secreted from 30 to 60 min of the perfusion period: This latter value was used to calculate secretory rates per unit endocrine pancreas.

Pancreas weight

Initially, estimates of pancreas weight were to have been obtained from each perfused, formalin-fixed pancreas that had been dissected free of fat and blotted dry. However, reweighings indicated that the blotting technique gave variable results. Because these tissues were required for morphometric studies, lyophilization of the pancreases was not possible. As a result, we obtained pancreas weights from five additional similarly treated animals from each experimental group. This was done by infusing the pancreas of each animal with formalin through the bile duct; the pancreas was subsequently isolated and immersed in formalin for 3 d. After this, extraneous fat and connective tissue were removed with the aid of a dissecting scope and the pancreatic tissue was frozen, lyophilized, and weighed. Mean pancreas weights

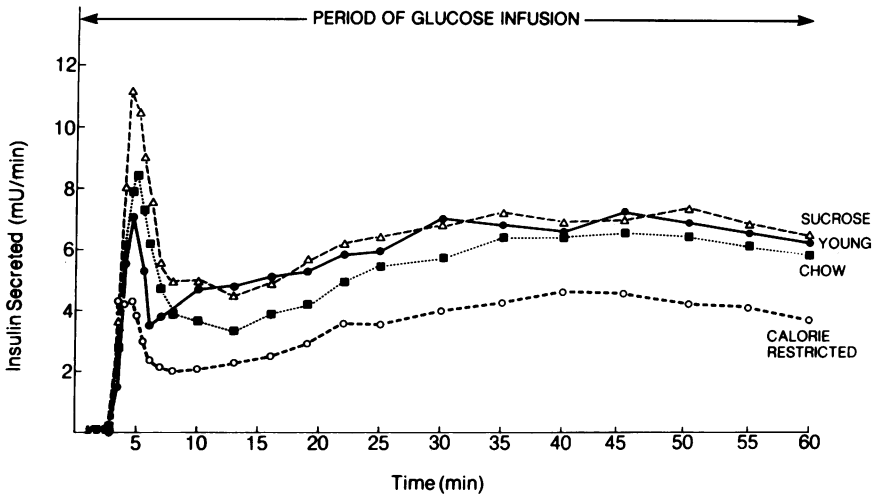

FIGURE 1 Effect of age and differences in amount and kind of calories on maximal pancreatic glucose-stimulated insulin release (young, ●-----●; 12-mo-old chow-fed, ■-----■; 12-mo-old sucrose-fed, Δ-----Δ; 12-mo-old calorie-restricted, O-----O). Pancreases of six animals from each category were perfused (5 ml/min) with glucose (at a concentration of 300 mg/dl from 0 to 60 min). Mean (±SEM) secretory rates for representative time points are given in Table I.

from each experimental category of rats were used to adjust individual pancreas secretion rates.

Morphometric studies

The splenic region (11) of each formalin-fixed pancreas used for the secretion studies was subsequently divided into horizontal slices that were processed and embedded in paraffin for hematoxylin eosin or Masson's trichrome stains. Using an eyepiece grid, volume density measurements were made on the composition of the islets as well as on the total islet content of the sections (1, 2). Measurements of islet content per pancreas and connective tissue content per islet were carried out at low magnification (×200) on one Masson's trichrome-stained section of each paraffin-embedded tissue slice. Such sections averaged 20 × 15 mm in size and contained profiles of 50–80 islets. All of these islets were included in volume density measurements; on the average, decisions as to islet content per pancreas or islet composition were based on 4,000–5,000 counts (grid intersections) per section.

RESULTS

As anticipated, rats fed either standard laboratory chow or a sucrose-rich diet of identical calorie content weighed the same at 12 mo of age, and both groups of rats were relatively obese: Mean (±SEM) body weight was 752±27 and 759±14 g, respectively for the chow- and sucrose-fed animals. In contrast, the calorie-restricted animals of the same age, weighed 300 g less; mean (±SEM) weight of these rats was 430±5 g.

Insulin secretion. Fig. 1 and Table I show maximal glucose-stimulated insulin release by the perfused pancreas of 2-mo-old rats fed standard chow and 12-mo-old rats fed one of three different diets for most of their lives. Qualitatively, the biphasic pattern of insulin release by each group of rats studied is similar, and corresponds to the normal biphasic secretory pattern described by Curry et al. (8, 9).

In these studies, the first 2 min of perfusion reflects basal rates of nonstimulated insulin release. Evidence of glucose-stimulated insulin release begins simultaneously in all groups by 3½ min, and peak first-phase secretion is seen within the next 60 s. This is followed in all cases by a more sustained second phase of secretion, which reaches maximal values ~30–35 min after the onset of secretion. Fig. 1 and Table I indicate that when stimulated in this fashion, the average pancreas from young (2-mo-old) rats and older (12-mo-old) rats fed chow or sucrose, secrete similar amounts of insulin. However, when given the same glucose challenge, the perfused pancreases of equally old (12 mo-old), calorie-restricted animals release approximately one-third less insulin at most time points measured. Table II gives average values for the total amount of insulin released in both phases of secretion

TABLE I
Mean±SEM Secretory Rates during Pancreatic Perfusion

Animals	Time		
	4½ min	30 min	45 min
Young	7.0±1.0	7.0±1.5	7.3±1.6
12-mo-old chow-fed	7.9±1.0	5.7±1.0	6.6±1.1
12-mo-old sucrose-fed	11.0±1.3	6.9±1.0	7.3±1.0
12-mo-old calorie-restricted	4.3±0.8	4.0±0.5	4.3±0.5

TABLE II

Effect of Age and Environmental Intervention on Total Pancreas First- and Second-Phase Insulin Secretion

Animals	First-phase insulin secretion 1–8 min	Second-phase insulin secretion 30–60 min
	mU	
2-mo-old chow-fed	23.7±4.2	238±55
12-mo-old chow-fed	30.7±3.9	215±37
12-mo-old sucrose-fed	37.6±8.2	246±44
12-mo-old calorie-restricted	17.3±2.0*	143±14‡

* $P < 0.05$ as compared with 12-mo-old chow or sucrose-fed rats.
‡ $P < 0.05$ as compared with 12-mo-old sucrose-fed rats.

by pancreases of rats in the various experimental categories. When these values are expressed per unit of total body weight, one finds that the young rats secrete 0.95 mU insulin/30 min per g body weight, whereas the 12-mo-old calorie-restricted chow- and sucrose-fed rats secrete 0.33, 0.29, and 0.32 mU insulin/30 min per g body weight, respectively.

The data in Fig. 1 and Tables I and II illustrate the insulin-secretory capacity of the entire pancreas. In order to express insulin secretion per unit beta cell mass, it is necessary to take into account both the size of the pancreas and the proportion of the pancreas occupied by beta cells. These calculations appear in Table III. Thus, column A again illustrates that maximal glucose-stimulated insulin release per pancreas is reduced only in the 12-mo-old calorie-restricted rats. However, when the secretory data from rats in the various categories of column A are adjusted for differences in pancreas weight (column B), a new picture emerges regarding the capacity of the pancreas of aging rats to secrete insulin (column C). These calculations indicate that maximal insulin release (per gram pancreas) in all three categories of aging animals averages about half that released from the pancreas of young animals. However, these figures have still not considered the fact that islet cell mass also changes in pancreases of aging animals (1, 2, 12). To take this into account, an estimate of the islet content of each pancreas was obtained from volume density measurements of each pancreas used in the perfusion studies. Islet content per volume pancreas was calculated and corrected for the amount of connective tissue present in the islets (2). This corrected figure for the various groups appears in column D, and, when multiplied by the mean weight of pancreases from similar rats, provides an estimate of the endocrine cell mass per total pancreas (column E). Finally, when the amount of insulin released from each pancreas is divided by the endocrine tissue mass of the same pancreas, an estimate of insulin secretion per islet cell mass can be obtained (column F). When this calculation is made, insulin secretion per unit endocrine pancreas of aging animals is found to be reduced by approximately the same amount in all the aging animals studied. As such, the decline in insulin-secretory capacity seen with age appears to be independent of animal weight and dietary history.

Additional morphologic observations. The pancreatic islets from the calorie-restricted rats were, as previously described (2), similar in structure and composition to islets from young animals. In contrast, islets from chow- and sucrose-fed animals were generally enlarged, multilobulated, and fibrotic (2). The amount of connective tissue infiltration within the islets varied widely among the different pancreases studied, but was especially prominent in the 12-mo-old sucrose-fed rats: i.e., mean (±SEM) values for islet connective tis-

TABLE III

*Insulin Secretion per Total Pancreas, per Unit Pancreas Weight, and per Unit Endocrine Pancreas**

Animals	A Insulin release per pancreas§	B Pancreas dry weight	C Insulin release per g pancreas	D Islet content of pancreas‖	E Islet cell mass¶	F Insulin release per μg islet cell mass
	mU/30 min	*g*	*mU/min*	*%*	*μg*	*mU/min*
2-mo-old chow-fed	238±55	0.27±0.01	29.4±6	0.6±0.1	1.7±0.3	4.7±1.4
12-mo-old calorie-restricted	143±14	0.34±0.02‡	14.0±1‡	1.0±0.2	3.6±0.8	1.3±0.4‡
12-mo-old chow-fed	215±37	0.48±0.03‡	14.9±3	1.4±0.2‡	6.3±1.0‡	1.1±0.3‡
12-mo-old sucrose fed	246±44	0.45±0.05‡	18.2±3	1.2±0.4	5.4±1.9‡	1.5±0.4‡

* Values are expressed as mean±SEM. In columns A, B, and D these values represent raw data: In columns C, E, and F the values are derived from individual animal data points, and corrected for pancreas size by using mean values for pancreas dry weight (see Methods).
‡ $P < 0.05$ as compared to 2-mo-old chow-fed rats.
§ Total second-phase insulin secretion from 30 to 60 min.
‖ Islet content = (islet volume density − islet connective tissue volume density) ÷ (pancreas volume density × 100).
¶ Islet cell mass = (pancreas dry weight, B) × (islet content of pancreas, D).

sue content was 5±1 in 2-mo-old chow-fed rats and 13±2, 25±4, and 41±8 percent of islet volume in 12-mo-old calorie-restricted, chow-, and sucrose-fed rats, respectively. Additional cytochemical and ultrastructural details of such islets will form the substance of a separate report.

DISCUSSION

The results presented demonstrate that maximal glucose-induced insulin secretion per unit endocrine pancreas declines to less than one-third of the original values as rats grow from 2 to 12 mo of age, despite the presence of adequate insulin stores within the pancreases of the older animals (1). The quantitative nature of this age-related decline in glucose-stimulated insulin release is remarkably similar to that obtained previously from isolated islets of similar aged rats (1, 2). Thus, we have an unequivocal answer to the first question posed, i.e., age leads to a decrease in glucose-stimulated insulin secretion per unit endocrine pancreas, and previous results obtained with isolated islets do not appear to have been an artifact of collagenase digestion.

Although the current results indicate that insulin secretion per unit endocrine pancreas declines with age, it is also apparent that the total pancreatic insulin secretory response can be maintained. Obviously, this can only occur in the older animals as the consequence of an increase in the mass of the endocrine pancreas, which in the case of the 12-mo-old chow- and sucrose-fed rats is accomplished by an approximate doubling of both pancreatic dry weight and proportion of the pancreas composed of islet tissue. Thus, the resultant three- to fourfold increase in mass of endocrine pancreas totally compensates for the decline in glucose-stimulated insulin secretion per unit islet cell mass. The current experiments were not designed to deal with the reason for the increase in endocrine pancreas mass, but previous data provide a fairly straightforward explanation. As rats grow older they become obese and physically inactive, and we have both indirect (2) and direct (6) evidence that such changes lead to a loss of normal in vivo insulin sensitivity. Under these conditions, the obese older animal is faced with the need to increase insulin secretion in order to maintain glucose homeostasis. Because the capacity of the average beta cell to secrete insulin actually declines with age, the 12-mo-old chow- or sucrose-fed, obese rat only can compensate by increasing its pancreatic beta cell mass. However, the degree to which the mass of the endocrine pancreas increases with age is probably a function of associated changes in in vivo insulin action. For example, a 12-mo-old, lean, relatively insulin-sensitive, calorie-restricted rat would not need to chronically increase its insulin output to the same extent as a more obese animal (2), and, as a consequence, would not be expected to have the same enlargement of islet cell mass as the more obese animal. As a result of this more limited amount of endocrine pancreas, the total pancreatic insulin secretory capacity of the 12-mo-old calorie-restricted animal would also be expected to be less. The data in Tables I and II provide evidence for these speculations, and suggest that the effect of age on total insulin secretory capacity can be modified by a change in mass of the endocrine pancreas, and that these structural changes are compensatory responses, secondary to age-related variations in in vivo insulin action. However, it should be pointed out that such considerations of the impact of in vivo insulin action on the endocrine cell mass need not be accepted in order to answer the second question posed. Indeed, the data demonstrate clearly that older rats can maintain maximal insulin secretion at the level of young rats, despite a decrease in response per beta cell, and that this is achieved by an increase in the amount of insulin secretory tissue in the pancreas.

If we now turn to the third issue raised, the results highlight the fact that environmental manipulations, which profoundly modify the impact of age on total insulin secretory capacity, have little effect on the ability of the individual beta cell to respond to insulin. Thus, maximal rates of glucose-stimulated insulin release per islet mass of perfused pancreases from all three groups of 12-mo-old rats are essentially equal, and represent less than one-third the response of the perfused pancreas of 2-mo-old rats. These results are very similar, both qualitatively and quantitatively to those previously published using isolated islets (1, 2), and strongly support our original suggestion that the age-related decline in insulin secretion is an inevitable function of the aging process. Insofar as these results may also apply to man, it seems obvious that efforts to prevent the decompensation of glucose tolerance that occurs with age (13–16) should be aimed at means of enhancing in vivo insulin action.

In summary, these experiments confirm the existence of the age-related decline in beta cell insulin secretion previously seen in collagenase-isolated islets, and show that this change in rats is a function of age per se, and is not influenced by variations in amount or kind of calories ingested. Furthermore, they suggest that obese and/or sucrose-fed 12-mo-old rats, who must contend with both this problem and the need to overcome an age-related loss of normal insulin sensitivity, accomplish this feat by expanding their pancreatic pool of beta cells. Although this compensatory effort seems to be successful in the 12-mo-old rats studied, it should be remembered that these rats have lived out less than half of their expected life span, and it is

not yet clear what impact further aging would have on these events. Indeed, one wonders if with increasing time and/or the continued challenge of a sucrose-rich diet (which leads to exceedingly fibrotic islets in 12-mo-old rats), the limits of the pancreas to compensate may not be exceeded. If so, pancreatic exhaustion may occur in old age, resulting in unmistakable glucose intolerance. Future studies will be directed toward examining this possibility.

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