Impaired insulin secretion of aging: Role of renal failure and hyperparathyroidism

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Impaired insulin secretion of aging: Role of renal failure and hyperparathyroidism. Available data indicate that insulin secretion is impaired with aging. Almost all the studies that examined insulin secretion by old animals did not take into consideration the state of renal function or the blood levels of parathyroid hormone (PTH). Old animals may have chronic renal failure (CRF) and secondary hyperparathyroidism, and both of these conditions impair insulin secretion. It is possible, therefore, that the impaired insulin secretion of aging is not due to old age *per se*, but rather to associated CRF and excess PTH. The present study examined this issue in adult (6 month old) and senescent rats (2 years old) with and without CRF and excess PTH. Senescent rats without CRF had normal renal function and normal blood levels of PTH, and the values were not different from those observed in adult rats. Creatinine clearance in senescent rats with CRF was significantly ($P < 0.01$) lower and serum levels of PTH were significantly ($P < 0.01$) higher than in senescent animals without CRF and than in the adult rats as well. Only the senescent rats with CRF displayed glucose intolerance during intravenous glucose tolerance test. For any given level of blood glucose, plasma insulin levels were lower in senescent rats with CRF than in the adult rat or senescent animals without CRF. Both initial phase (139 ± 45 pg/islet · 8 min) and total (808 ± 216 pg/islet · 33 min) insulin secretion from pancreatic islets of the senescent rats with CRF and excess PTH were significantly lower than those in senescent rats with normal renal function (658 ± 117 pg/islet · 8 min and 3294 ± 290 pg/islet · 33 min, respectively) or in adult rats (710 ± 134 pg/islet · 8 min and 3183 ± 366 pg/islet · 33 min, respectively). There were no significant differences in insulin secretion between the adult rats and the senescent ones with normal renal function. The data demonstrate that the impaired insulin secretion by the pancreatic islets in old rats is not necessarily related to the higher age *per se*, but is due to the associated CRF and secondary hyperparathyroidism that develops in many, but not all old animals. Our results indicate that studies examining the effect of aging on body function should take into consideration the level of renal function and of the serum PTH, since both CRF and excess PTH adversely affect the functional integrity of many organs.

Abnormalities in glucose metabolism are commonly encountered in aged humans [1–3] and animals [4–6]. It is generally accepted that aging is associated with impaired responsiveness to the peripheral action of insulin [1–3], most likely due to a postreceptor defect [7]. However, the data on the effects of

aging on other aspects of glucose homeostasis are not uniform. Indeed, normal [3, 7] or decreased [1–10] metabolic clearance rate of insulin and impaired [4–6, 11–13] or normal [1, 14, 15] insulin secretion were reported in old humans or animals. The reasons for this variability are not evident.

The combination of peripheral resistance to the action of insulin secondary to a postreceptor defect, decreased metabolic clearance of insulin, and impaired insulin secretion are the hallmarks of glucose intolerance of chronic renal failure (CRF) [16]. CRF is associated with secondary hyperparathyroidism and elevated blood levels of parathyroid hormone (PTH) [17], and chronic exposure to excess PTH in the absence [18] or presence of CRF impairs glucose-induced insulin secretion [19–21]. It is of interest that in the studies dealing with the effect of aging on glucose homeostasis, the level of renal function and the presence or absence of hyperparathyroidism have not been evaluated nor considered in the interpretation of the results.

Both humans [22] and animals [23] may lose renal function to a variable degree and may have secondary hyperparathyroidism [24–27] with aging. Consequently, in an aged population of humans or animals, one may find renal function to be normal, moderately impaired, or even markedly reduced with or without secondary hyperparathyroidism. Therefore, in order to evaluate the effect of aging on glucose homeostasis, one must study an aged population with normal renal function and without elevated blood levels of PTH, and compare it with one in which renal function is impaired and secondary hyperparathyroidism is present.

This study examined glucose-induced insulin secretion by pancreatic islets obtained from two groups of 24 month old rats with and without CRF and secondary hyperparathyroidism. Six month old rats with normal renal function and normal blood levels of PTH served as controls.

Methods

Male Wistar rats weighing 430 to 600 g were obtained from the aging colony of the Gerontology Research Center, National Institute of Aging, Baltimore, Maryland, USA. Two groups of rats were studied: a) six months old rats (adult rat) weighing 506 ± 13 g and b) twenty-four month old rats (senescent rats) with (weight 488 ± 16 g) and without (weight 508 ± 12 g) renal failure and secondary hyperparathyroidism. It is noted that kidney

disease (glomerulosclerosis and tubular atrophy) occurs in a significant number of these rats belonging to this colony [28]. In our studies, senescent rats were considered to have renal failure if their serum creatinine concentrations were twice and their creatinine clearances were one half those of the six month old animals, and to have secondary hyperparathyroidism when their blood levels of PTH were more than twice those of the six month old rats. Senescent rats which had values of serum creatinine, PTH, and creatinine clearances not different from those in the six month old animals were considered to have normal renal function and no secondary hyperparathyroidism.

All rats were kept throughout their entire lives at room temperature (22 to 24°C) with a photoperiods of 12 hours light and 12 hours dark, and were maintained on standard National Institutes of Health rat chow. This diet contains 25.3% protein, 1.2% calcium and 0.9% phosphorus. The rats were transferred to our laboratory 7 to 10 days prior to the study and were maintained at the same conditions. The animals were housed in metabolic cages for collection of 24 hour urine samples.

One hour intravenous glucose tolerance test (IVGTT) was done in the three groups of rats. The day before the study all rats were weighed and anesthetized with intraperitoneal injection of 40 to 50 mg phenobarbital/kg body wt (Western Medical Supply, Arcadia, California, USA). Through an incision of the ventral surface of the neck, the jugular vein and carotid artery were cannulated with PE-10 tubing filled with a solution of sodium heparin (1000 IU/ml in 0.9% saline). The tubing was led subcutaneously to emerge at the base of the neck. After surgery, the animals were housed in individual cages and given regular diet and water. IVGTT was performed the following day after 12 hours of fasting and while the rats were awake. The animals received 0.5 g of D-glucose/kg body wt as an intravenous bolus injection. A total of six blood samples of 100 μl each were collected serially before and at 5, 15, 30, 45, and 60 minutes after the glucose injection from the arterial line for measurement of glucose and insulin.

In another group of rats, insulin secretion from pancreatic islets was examined. On the day of the experiments, the rats were decapitated and the pancreata were removed, trimmed free of adipose tissue and minced coarsely in ice-chilled Hank's solution. The islets of Langerhans were isolated by the collagenase digestion method [29]. Their size was measured under dissecting microscope. Glucose-induced insulin secretion was evaluated by dynamic studies using a four channel perifusion apparatus as described previously from our laboratory [20]. Twenty-five size-matched islets approximately 250 to 300 μm in diameter were placed in each of the four conical chambers of 0.07 ml capacity, and were perifused at a rate of 0.8 ml/min with the incubation media containing 2.8 mM D-glucose at a temperature of 37°C and a gas mixture of 95% O_2 and 5% CO_2. The incubation media was a modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM HEPES and 0.5 g/dl bovine serum albumin. After leaving the chambers, the perifusate was filtered through 8.0 μm pore size filter (Sartorius, Burlingam, California, USA) and was collected. Each study was performed in duplicates. After 39 minutes of pre-incubation, the collection of the effluent was started and continued at a one minute interval for 41 minutes. The first six collections (6 min) represented the basal level of insulin during perifusion with 2.8 mM D-glucose. Thereafter, the D-glucose concentration in the peri-

fusate was increased to 16.7 mM and an additional 35 samples were collected. Insulin concentration was then determined in the various samples of the effluent by the charcoal coated immunoassay using rat insulin as standard [30].

The changes in insulin secretion from baseline over time were evaluated by calculating the area under the curve for each study utilizing the trapezoidal rule. Insulin release from the islets started to increase two minutes after the change in the concentration of D-glucose in the perifusate to 16.7 mM. The average value of the insulin release prior to that (8 min) was used as the basal insulin release in response to 2.8 mM D-glucose. Area under the curve in the first eight minutes (minute 2 to 10) after the exposure of the islets to 16.7 mM D-glucose was calculated and considered to be the initial phase of insulin secretion. The total insulin release was calculated for 33 minutes (minute 2 to 35). The area under the curve of insulin release and the area under the curve for plasma glucose during IVGTT in each of the various groups of rats as well as the different biochemical data were compared with each other by one way analysis of variance using the Duncan multiple range test.

In another set of rats (3 in each groups) the pancreata were fixed in 10% (buffered) formaldehyde and embedded in paraffin after routine processing in a tissue processor. Sections were cut at a 5 micrometer. One section from each rat was stained with hematoxylin and eosin. A parallel section was stained by immunoperoxidase technique using an antibody against insulin.

Urine creatinine was measured with a creatinine kit from Stanbio Laboratory, San Antonio, Texas, USA; serum levels of creatinine and phosphorus were determined with a Technicon autoanalyzer (Technicon Instruments Inc., Tarrytown, New York, USA) and serum concentrations of calcium and magnesium were estimated with a Perkin Elmer atomic absorption spectophotometer model 503 (Perkin Elmer Corp., Norwalk, Connecticut, USA). PTH levels in serum were determined by a INS-PTH immunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, California, USA). This assay recognizes the aminoterminal fragment of PTH. The assay showed highly reproducible IC50 for inhibition of ^{125}I-PTH binding of 21.4 ± 0.8 pg and less than 10% differences within an assay. A sample volume of 120 μl was used for most of the assays. Dilutions were made when necessary and the final result was based on the lowest dilutions.

Results

Table 1 presents the weight and the biochemical data in the three groups of animals. The weight of the senescent rats with or without CRF or secondary hyperparathyroidism was not different from that of the adult rats. The concentrations of BUN, plasma levels of creatinine, calcium, and magnesium and serum PTH and the value of creatinine clearance in the 24 month old rats without CRF were not different from those in the 6 month old rats. In contrast, the 24 month old rats with CRF had significantly ($P < 0.01$) higher levels of BUN, plasma creatinine and serum PTH, and lower values of creatinine clearance than the 24 month old rats without CRF and the 6 month old rats as well; the concentrations of plasma calcium and magnesium in these two groups of senescent rats were not different. The concentrations of plasma phosphorus in the 24 month old rats with or without CRF were significantly ($P < 0.01$) lower than the values in the 6 month old rats. This

Table 1. Biochemical data and insulin secretion from pancreatic islets in adult (6 months old) and in senescent (24 month old) rats with and without chronic renal failure and secondary hyperparathyroidism

| | Body weight | Plasma levels *mg/dl* | | | | | Serum 1-34 PTH | Creatinine clearance | Insulin secretion *pg/islet* | |
| | | | | | | | | | Early | Total |
	g	BUN	Cr	Ca	P	Mg	*pg/ml*	*μl/min/ 100 g*	8 min	33 min
1. Six month old (N = 11)	506 ± 13	29 ± 1.7	0.39 ± 0.02	10.4 ± 0.23	7.1 ± 0.25	2.4 ± 0.07	39 ± 2.9	554 ± 54	710 ± 134	3813 ± 366 (N = 6)
2. Twenty-four months old										
a. Without CRF (N = 13)	508 ± 12	36 ± 4.8	0.49 ± 0.02	10.1 ± 0.07	5.3 ± 0.18[a]	2.5 ± 0.12	41 ± 5.9	432 ± 62	658 ± 117	3294 ± 290 (N = 6)
b. With CRF and 2° HPTISM (N = 12)	488 ± 16	80 ± 8.2[a]	1.18 ± 0.09[a]	10.4 ± 0.21	5.2 ± 0.21[a]	2.8 ± 0.14	112 ± 17.9[a]	204 ± 23[a]	139 ± 45[a]	808 ± 216[a] (N = 7)

Data are presented as mean ± SE. Abbreviations are: BUN, Blood urea nitrogen; Cr, creatinine; Ca, calcium; P, phosphorus; Mg, Magnesium; CRF, chronic renal failure; 2° HPTISM, secondary hyperparathyroidism.
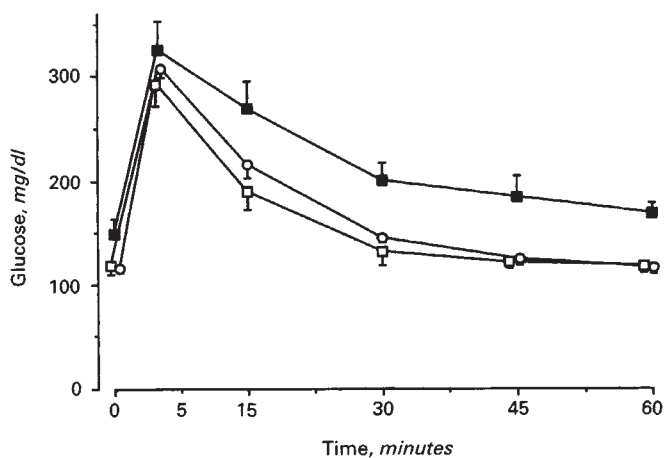[a] $P < 0.01$ from other groups

Fig. 1. *The changes in the plasma levels of glucose during intravenous glucose tolerance test performed in the three groups of animals:* (O) *six months;* (□) *24 months − CRF;* (■) *24 months + CRF. Each datum point represents mean value of 5 to 7 experiments and brackets denote 1 SE.*
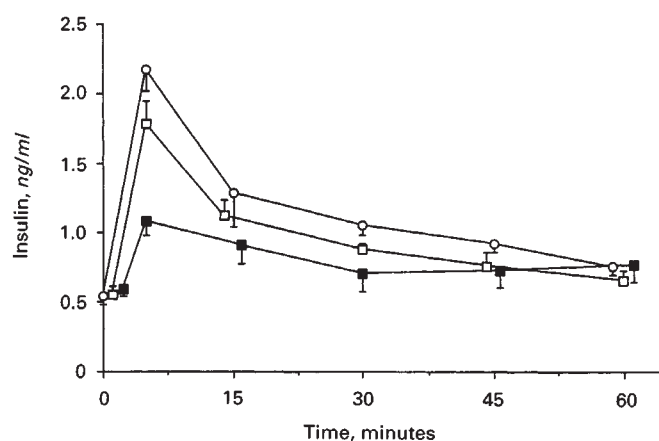

Fig. 2. *The changes in plasma insulin levels during intravenous glucose tolerance test performed in the three groups of animals.* Symbols are: (O) 6 months; (□) 24 months − CRF; (■) 24 months + CRF. Each datum point represents mean value of 5–7 experiments and brackets denote 1 SE.

observation is similar to previously reported data on the concentration of serum phosphorus in old Wistar rats [28].

Figure 1 shows the changes in the plasma levels of glucose during IVGTT in the three groups of animals. The plasma levels of glucose reached their peak within five minutes after the injection of glucose load and decreased thereafter. The senescent rats with CRF and secondary hyperparathyroidism displayed glucose intolerance with the area under the curve for their plasma glucose (13157 ± 1103 mg/dl · 60 min) being significantly ($P < 0.05$) higher than that in the senescent rats without CRF (9530 ± 530 mg/dl · 60 min) or the adult rats (10178 ± 180 mg/dl · 60 min). The values in the latter two groups were not different. There were significant increments in plasma insulin levels in adult and senescent rats without CRF but only small changes in senescent rats with CRF and secondary hyperparathyroidism (Fig. 2). Indeed for any given plasma glucose levels, the plasma insulin levels were higher in the adult

and senescent rats without CRF than in the senescent rats with CRF and secondary hyperparathyroidism (Fig. 3).

Both the initial phase and the total amount of glucose-induced insulin secretion by pancreatic islets from 24 month old rats without CRF or secondary hyperparathyroidism were not different from the corresponding values observed in the 6 month old rats (Table 1, Fig. 4). In contrast, the senescent rats with CRF and elevated serum levels of PTH displayed marked and significant ($P < 0.01$) reductions in the amount of insulin secreted in the initial phase of the glucose-induced insulin release as well as in the total amount of insulin secreted during the 33 minutes of perifusion studies (Table 1, Fig. 4).

Figure 5 shows the histology of the pancreatic islets from adult and senescent rats with and without CRF. In all three groups of animals, the islets were normal in size, distribution, cellularity and structure, and there were no evidence of fibrosis or lymphocytic infiltration. Immunoperoxidase stain for insulin
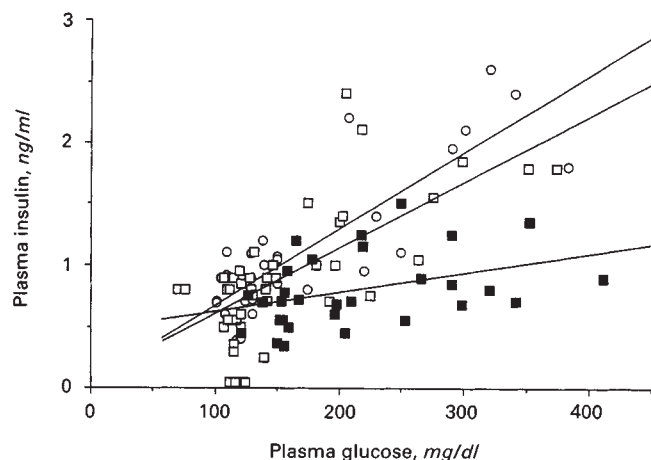
Fig. 3. *The relationship between plasma levels of insulin and glucose observed during the intravenous glucose tolerance test performed in the three groups of animals.* Symbols are: (○) six months, (□) 24 months without CRF, (■) 24 months with CRF. One animal of senescent rats with CRF had insulin levels of 1 or more ng/ml. It is this animal which makes the data of CRF rats appear to overlap with the data of the other two groups of rats. Without this one animal, the separation between insulin levels of old rats with CRF and the two groups of rats is more clear.

Fig. 4. *Dynamic glucose-induced insulin secretion by perifused pancreatic islets isolated from six adult (6 months old) rats (shaded area), six senescent (24 months old) rats with CRF and secondary hyperparathyroidism (■) and seven senescent (24 months old) rats without CRF or secondary hyperparathyroidism (□). The shaded area encompasses the mean value ± 1 SE. Each datum point represents mean value and brackets denote ± 1 SE.*

showed that 70 to 80% of the cells in the islets from the three groups of animals were beta cells that stained positively for cytoplasmic insulin.

Discussion

The results of the present study demonstrate that glucose intolerance is present in senescent rats with CRF and secondary hyperparathyroidism, but not in senescent rats without CRF and with normal levels of plasma PTH. Also, the plasma levels of insulin during the IVGTT in the senescent rats with CRF and excess PTH did not increase as much as in the other two groups of rats. Indeed, for any given plasma levels of glucose, the plasma insulin levels were lower in the senescent rats with CRF than in the senescent rats without CRF or in adult rats. These observations are consistent with reduced glucose-induced insulin secretion in senescent rats with CRF. The results of the perifusion studies of the pancreatic islets provide definite support for this notion in that islets from senescent rats with CRF and elevated blood levels of PTH displayed a marked and significant reduction in the initial phase as well as in the total amount of glucose-induced insulin secretion. This is in contrast to the normal glucose-induced insulin secretion by pancreatic islets of senescent rats without CRF and normal plasma levels of PTH.

Although the area under the curve for insulin during the IVGTT in old rats with CRF is significantly lower than in adult and old rats without CRF, the plasma insulin levels at 15, 30, 45 and 60 minutes were not significantly different. The reason for this latter finding is, most likely, due to different rates of insulin clearance. In the adult and old rats without renal failure, insulin levels declined faster than in old rats with CRF since the latter impairs insulin degradation [31]. Despite the small differences in plasma insulin levels at 15, 30, 45 and 60 minutes among the three groups of animals, the old rats with CRF had hypergly-
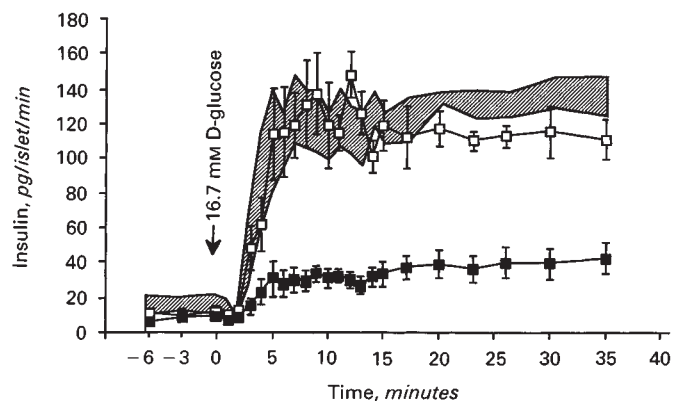
cemia. Further, hyperglycemia was also present at baseline in these animals despite plasma insulin levels similar to those in adult rats and old rats without CRF. These observations strongly suggest that old rats with CRF and excess PTH have peripheral resistance to the action of insulin.

The differences in glucose-induced insulin secretion between the islets of the two groups of senescent rats could not be attributed to variations in their weight, differences in protein intake, the concentration of serum calcium, phosphorus and magnesium, or the histology of the pancreatic islets since there were no significant differences between these parameters.

It has been shown that the pancreas of old rats contains greater number of large islets [32–35]. Kitahara and Adelman [36] have demonstrated that large islets secrete more insulin than smaller islets irrespective of age of the rat. In order to avoid the effect of islet size on insulin secretion by different groups of rats, one must exercise great caution in matching the size of the islets used for the study. We indeed matched the size of the islets obtained from our adult rats and the two groups of senescent rats. Therefore, the differences in insulin secretion observed in the rats of our study could not be accounted for by differences in islet size.

Our observations indicate that an impairment in insulin secretion that may occur with aging [4–6, 11–13] is not necessarily related to the higher age, but is most likely secondary to the effect of associated disease processes, such as CRF and secondary hyperparathyroidism, that may develop with aging [22–27]. Indeed, several lines of evidence indicate that even in adult or young animals, CRF impairs glucose-induced insulin secretion, and this effect is due to the secondary hyperparathyroidism of CRF [18–21]. Akmal et al, utilizing hyperglycemic clamp studies, showed that insulin secretion in adult dogs with CRF is impaired [19], and Fadda et al [20, 21] demonstrated that glucose-induced insulin secretion by pancreatic islets isolated from young rats with CRF displayed marked reduction in both the initial phase and the total amount of insulin secretion. This defect in insulin secretion was corrected by prior parathyroidectomy [19–21]. Mak et al [37] confirmed these observations in
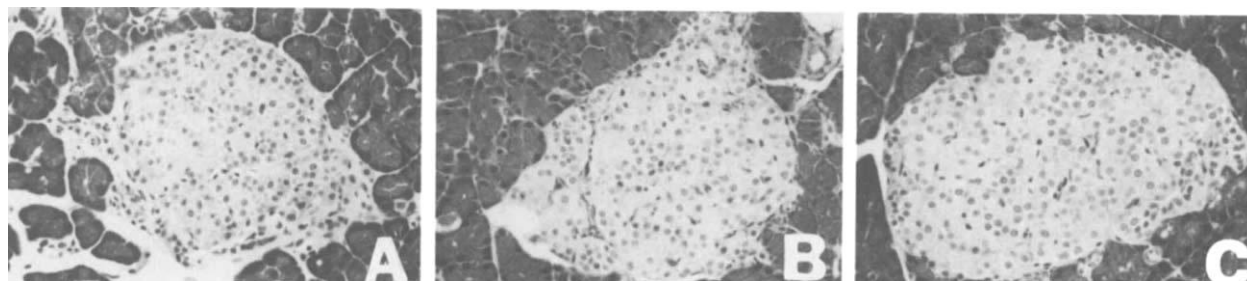
Fig. 5. *Light microscopy of histological sections of islets from adult rats* (**A**)*, senescent rats without CRF* (**B**) *and senescent rats with CRF and secondary hyperparathyroidism* (**C**)*,* (H & E × 100).

children with CRF. In addition, Perna et al [18] reported that insulin secretion by pancreatic islets isolated from young rats with normal renal function but treated with PTH for six weeks is impaired. Thus, it is apparent that chronic excess of PTH in the presence or absence of CRF impairs glucose-induced insulin secretion.

It is of interest that Molina, Premdas, and Lipson [13] found that while glucose-induced insulin release by islets from old rats is impaired, glyceraldehyde-induced insulin secretion is normal. These observations are consistent with the notion that an abnormality in glucose metabolism at or after the level of glucokinase and before the level of triose phosphate is present in the islets of old rats. Similar observations were found in young rats with CRF [21] and in young normal rats treated with PTH [18]. In these animals glucose-induced insulin release was impaired but glyceraldehyde-induced release was normal.

All these observations taken together and the results of the present study strongly support the proposition that the impairment in insulin secretion of aging is, at least, partly due to the state of renal failure and the associated secondary hyperparathyroidism that is frequently present in aged humans and animals [22–27].

The mechanisms responsible for the impaired insulin secretion by islets from young rats with excess PTH in the presence or absence of CRF have already been studied in detail [18, 21]. These include a significant rise in resting levels of cytosolic calcium, a decrease in basal levels of ATP, a small increment in islet ATP in response to glucose, and a defect in glucose metabolism of the islet. Although these parameters were not examined in the present study, it is reasonable to suggest that islets from old rats with CRF and secondary hyperparathyroidism display similar cellular derangements as islets from young rats with CRF and elevated blood levels of PTH.

Acknowledgments

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