Insulin release from pancreatic islets: Effects of CRF and excess PTH

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Insulin release from pancreatic islets: Effects of CRF and excess PTH. Insulin secretion may be impaired in chronic renal failure (CRF) and available data suggest that this abnormality may be related to the state of secondary hyperparathyroidism of renal failure. We directly measured insulin release from isolated islets of Langerhans obtained from normal rats, CRF-control and CRF-PTX (parathyroidectomized) rats, and parathyroid hormone (PTH)-treated animals. Both early and total glucose-induced insulin release from islets of CRF-control were markedly and significantly ($P < 0.01$) lower than from islets of normal rats. Insulin release from islets of CRF-PTX rats was significantly ($P < 0.01$) higher than that from islets of CRF-control rats, and not different from insulin release from islets of normal rats. Forskolin and IBMX, which cause a rise in cAMP, significantly stimulated glucose-induced insulin release from islets of normal, CRF-control and CRF-PTX rats, but the increments from baseline were not significantly different between the three groups. Both early and total insulin release from islets obtained from PTH-treated rats with normal renal function were markedly and significantly ($P < 0.01$) lower than values obtained from normal rats. Calcium contents of the pancreas of CRF-control and PTH-treated rats were significantly ($P < 0.01$) higher than that in pancreas of normal rats and CRF-PTX animals, and values in the latter two groups of animals were not significantly different. The results show that: 1) CRF impairs insulin release from pancreatic islets; 2) this abnormality is reversed by prior parathyroidectomy; and 3) hyperparathyroidism induced by PTH-treatment in normal rats impairs insulin release from pancreatic islets. The data provide a direct evidence for the role of secondary hyperparathyroidism in the genesis of abnormal carbohydrate metabolism in CRF. This effect of excess PTH is not related to alterations in cAMP production but may potentially be due to calcium accumulation in the pancreas.

Available data in dogs [1] indicate that glucose intolerance does not develop in chronic renal failure (CRF) in the absence of parathyroid hormone (PTH). Also studies in uremic or dialyzed children [2], adolescents [3], and adults [3, 4] demonstrated that medical suppression of the parathyroid gland activity or parathyroidectomy was followed by normalization of the glucose intolerance. In studies utilizing hyperglycemic clamps [1–3], the absence of PTH or the normalization of its blood levels resulted in a significant increase in the insulin response to hyperglycemia without a change in the peripheral resistance to

insulin. It was, therefore, concluded that excess blood levels of PTH may interfere with the ability of the islets of Langerhans to release adequate amounts of insulin [1–4] to overcome the insulin resistant state usually present in uremia [5].

However, there is no direct evidence that the high blood levels of PTH indeed impair insulin release. Further, in the intact organism many other factors such as serum levels of calcium, phosphorus, magnesium or potassium may modulate the response of the pancreas to hyperglycemia. To further elucidate the interaction between CRF, PTH and insulin release, we examined insulin secretion by islets of Langerhans isolated from CRF rats with intact parathyroid glands, parathyroidectomized CRF rats, rats with normal renal function and a state of hyperparathyroidism produced by prolonged administration of PTH and control animals.

Methods

Sprague-Dawley rats weighing 320 to 375 g were studied. They were fed normal rat chow diet (ICN Nutritional Biochemical, Cleveland, Ohio, USA) throughout the study and allowed to drink at libitum.

Two types of protocols were used. In the first protocol, the rats were studied after 42 days of chronic renal failure in the presence and absence of the parathyroid glands. Parathyroidectomy (PTX) was performed by electrocautary, and the success of the procedure was ascertained by a decrease in serum levels of calcium of at least 2 mg/dl. The PTX rats were allowed to freely drink water containing 5% of calcium gluconate. This procedure is adequate to normalize plasma calcium in the PTX rats. Seven days after PTX, the animals underwent a right nephrectomy through a flank incision; four days later, a partial left nephrectomy was performed. The nephrectomy procedure was also done in rats with intact parathyroid glands. This protocol, therefore, provided two groups of animals with CRF: one with intact parathyroid glands (CRF-control) and the other without parathyroid glands (CRF-PTX).

In the second protocol, normal rats received intraperitoneal injection of 1-84 PTH (Sigma Chemical Company, St. Louis, Missouri, USA) for 42 days. The hormone was dissolved in normal saline and 50 μg was injected in the morning and another 50 μg in the late afternoon. The control animals received sham injections of the vehicle only.

Table 1. Early and total insulin release from pancreatic islets after various periods of parathyroid hormone treatment and chronic renal failure

	Serum creatine mg/dl	Insulin release pg/islet	
		Early (6 min)	Total (31 min)
4 day			
Control	0.53 ± 0.03	249 ± 36	3777 ± 479
PTH-treated	0.56 ± 0.04	279 ± 27	3077 ± 274
P	NS	NS	NS
10 day			
Control	0.51 ± 0.06	201 ± 87	3376 ± 201
PTH-treated	0.47 ± 0.02	287 ± 98	4490 ± 333
P	NS	NS	<0.05
21 day			
Control	0.52 ± 0.04	275 ± 62	3421 ± 261
PTH-treated	0.40 ± 0.05	208 ± 35	3152 ± 317
P	NS	NS	NS
Control	0.43 ± .03	305 ± 80	3581 ± 329
CRF-control	1.28 ± .12	243 ± 99	2345 ± 499
P	<0.01	NS	<0.05

Data are presented as mean ± SE of 4 studies.
Data of insulin release represent area under the curve ± SE.

The animals were sacrificed by decapitation and the pancreas was removed, dissected free of adipose tissue and lymph nodes. Islets of Langerhans were isolated by the collagenase digestion method of Lacy and Kostianovsky [6] and picked free of exocrine tissue under a dissecting microscope. With every study of CRF-control, CRF-PTX or PTH-treated rats a simultaneous study was performed in a normal animal when it was technically feasible. The insulin release from islets of the CRF-control and CRF-PTX were evaluated under static and dynamic conditions and dynamic studies were performed on islets from PTH-treated rats. Both the static and dynamic studies were done according to methods previously reported [7, 8].

Briefly, in the static studies the islets were preincubated for a period of 30 minutes at 37°C in a modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM HEPES and 0.5 mg/dl bovine serum albumin (incubation media) and 2.8 mM D-glucose. The islets were then matched for size by visual inspection and groups of 10 islets were incubated in tubes containing 1.0 ml of the incubation media and were studied with the following secretagogues: a) 2.8 mM D-glucose; b) 16.7 mM D-glucose; c) 100 μM isobutyl-l-methylxanthine (IBMX) and 16.7 mM D-glucose; and d) 10 μM forskolin and 16.7 mM D-glucose. After 30 minutes of incubation in a shaker bath at 37°C, the supernatants were aspirated for determination of insulin.

The dynamic studies were conducted in a four channel perifusion apparatus utilizing previously described methods [8]. Twenty-five size-matched islets 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 at 37°C and a gas mixture of 95% O_2 and 5% CO_2 being continuously bubbled into the perifusate. After leaving the chambers, the perifusate was filtered through 8.0 μm pore size filter (Sartorius, Burlingame, California, USA) and was collected. Each study was performed in duplicates. After 39 minutes of preincubation, the collection of the effluent
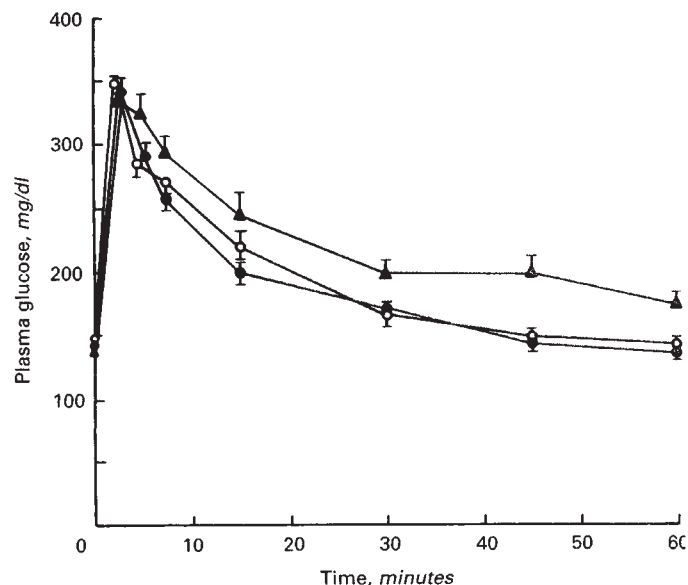

Fig. 1. *The changes in plasma glucose concentration during intravenous glucose tolerance test performed in 4 control rats (○), 5 CRF-control rats (▲), and 5 CRF-PTX animals (●). Each data point represents the mean value and the bracket 1 SE.*

was started and continued at a one minute interval for 41 minutes. The first six collections (6 minutes) represented the basal level of insulin release during perifusion with 2.8 mM D-glucose. Thereafter, the D-glucose concentration in the perifusate was increased to 16.7 mM and an additional 35 samples were collected and insulin concentrations were determined in various samples of the effluent.

Calcium content of the pancreas was measured. About 0.5 to 1.0 g of pancreas was placed in tared porcelain crucibles and weighed to 0.01 mg. All samples were dried at 105°C for 48 hours and then reweighed to determine water content. Samples were then ashed for 12 hours in an oven with 700°C. The samples were extracted in 0.75 N HNO_3 for 24 hours and calcium concentration was determined.

One hour intravenous glucose tolerance test was also done in control, CRF-control and in CRF-PTX rats. The animals were not fasted before the test. The jugular vein and carotid artery were cannulated with PE 10 tubing under general anesthesia with Ketamine-HCl 25 mg/kg (Bristol Laboratories, Syracuse, New York, USA). The animals were allowed to recover from the surgical procedure and were studied eight hours later in the awake state. The rats received 0.5 g of D-glucose /kg body wt in a bolus intravenous injection. A total of eight blood samples of 60 μl were collected serially from the arterial line for the measurement of glucose.

In the dynamic study of insulin release, the changes from baseline with time were examined by calculating the area under the curve for each study. Insulin release started to increase four minutes after the change of the concentration of D-glucose in the perifusate to 16.7 mM. Therefore, the average values of insulin release during the six minutes prior to the change in glucose concentration and of the four minutes immediately thereafter were used as a baseline level. The calculation of area under the curve allowed us to estimate insulin release during the
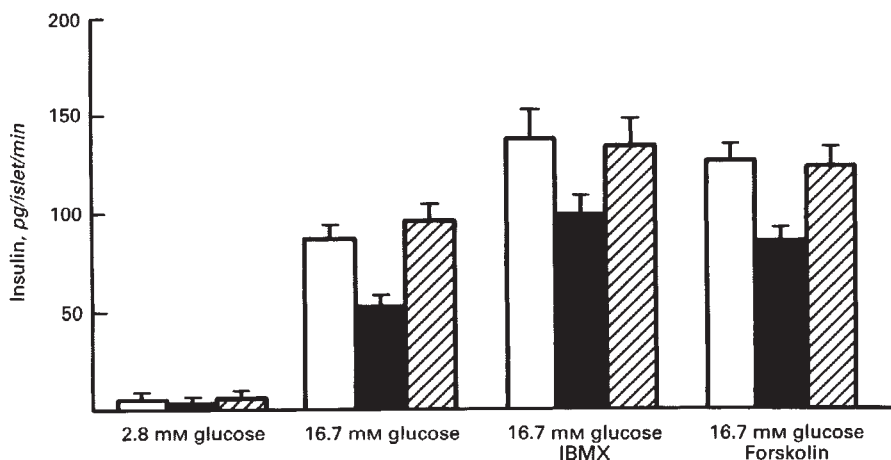
Fig. 2. *Insulin release from pancreatic islet during studies with static incubation in 13 normal rats (□), 6 CRF-control rats (■) and 6 CRF-PTX rats (▨). Each column represents mean value and brackets 1 SE.*
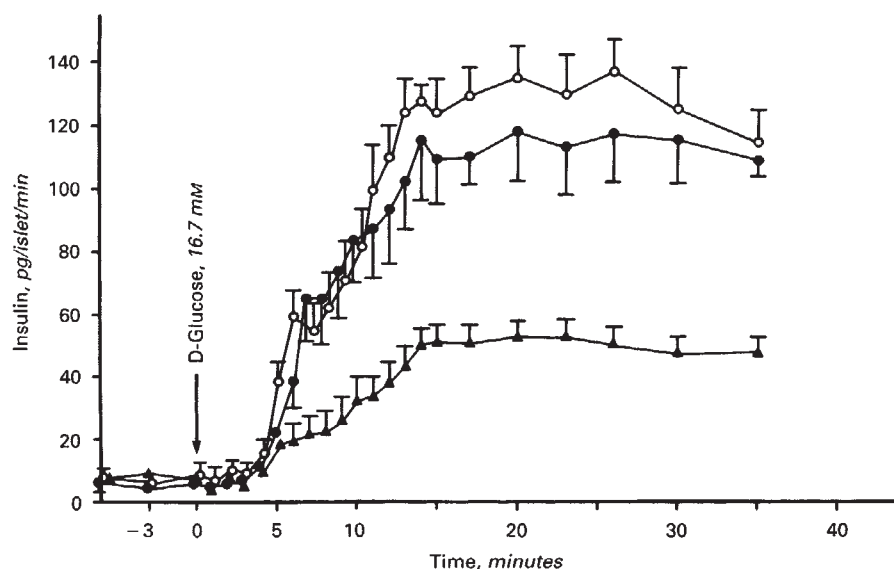

Fig. 3. *Dynamic insulin release from perifused pancreatic islets in 5 control rats (○), 5 CRF-control animal (▲) and 5 CRF-PTX rats (●). Each data point represents the mean value and brackets 1 SE.*

initial phase (5 min between min 4 to 9) and the total insulin release (31 min between min 4 to 35).

The choice to perform the study after six weeks of PTH administration or of CRF was based on results of experiments carried out to determine the time relationship between the duration of CRF or PTH treatment and insulin release from pancreatic islets. We found that PTH treatment of 4, 10 and 21 days did not produce significant reduction in insulin release and that the effects of 21 days of CRF were variable with only the mean value of total insulin release being modestly significantly ($P < 0.05$) lower than control. These data are shown in Table 1. We therefore used the six weeks protocol where the effects on insulin release was consistent and marked.

The measurement of calcium and magnesium was made with Perkins Elmer atomic absorption spectrophotometer, model 503 (Perkin Elmer Corp., Norwalk, Connecticut, USA), those of creatinine and phosphorus with Techincon autoanalyzer (Techincon Instrument Inc., Tarrytown, New York, USA), and those of glucose by glucose oxidase method utilizing Beckman glucose analyzer II (Beckman Instruments, Inc., Fullerton,

California, USA). Insulin was determined by charcoal-coated radioimmunoassay [9] using rat insulin as standard.

Statistical analysis was done with the Clinfo computer system. The data are presented as mean ± SE. Changes from base line in parameters with multiple measurements with time (glucose tolerance and dynamic insulin release) were evaluated by calculating area under the curve for each experiment utilizing the trapezoidal rule. The areas under the curve as well as the data from studies of static insulin release were analyzed by one-way analysis of variance and compared with each other using the Duncan multiple range test. Non-parametric analysis was also done using Wilcox non-paired rank sum tests adjusted for multiple comparison. The area under the curve for the studies from control and PTH-treated rats were compared by unpaired *t*-test.

Results

Intravenous glucose tolerance

Figure 1 depicts the changes in blood glucose during intravenous glucose tolerance tests in control, CRF-control and CRF-

Table 2. The biochemical data, insulin release during static studies and calcium content of pancreas in control, CRF-control an CRF-PTX rats

	Body weight g	B Cr	B Ca	B P	B Mg
			mg/dl		
a. Control N = 13	356 ± 6	0.55 ± 0.04	9.2 ± 0.2	6.9 ± 0.3	1.7 ± 0.07
b. CRF-Control N = 6	343 ± 5	1.90 ± 0.08	9.3 ± 0.4	6.4 ± 0.94	2.7 ± 0.3
c. CRF-PTX N = 6	341 ± 10	1.79 ± 0.05	9.3 ± 0.3	5.1 ± 1.3	2.8 ± 0.2
P values					
a vs. b	NS	<0.01	NS	NS	<0.05
a vs. c	NS	<0.01	NS	NS	<0.05
b vs. c	NS	NS	NS	NS	NS

Abbreviations are: B, blood; Cr, creatinine; Ca, calcium; P, phosphorus; and Mg, magnesium. Data are presented as mean ± SE. Δ1, increment of IBMX-induced insulin release above 16.7 mM glucose release. Δ2, increment of forskolin-induced insulin release above 16.7 mM gluocse release.
[a] The N for control is 6, for CRF control is 5 and for CRF-PTX is 6.
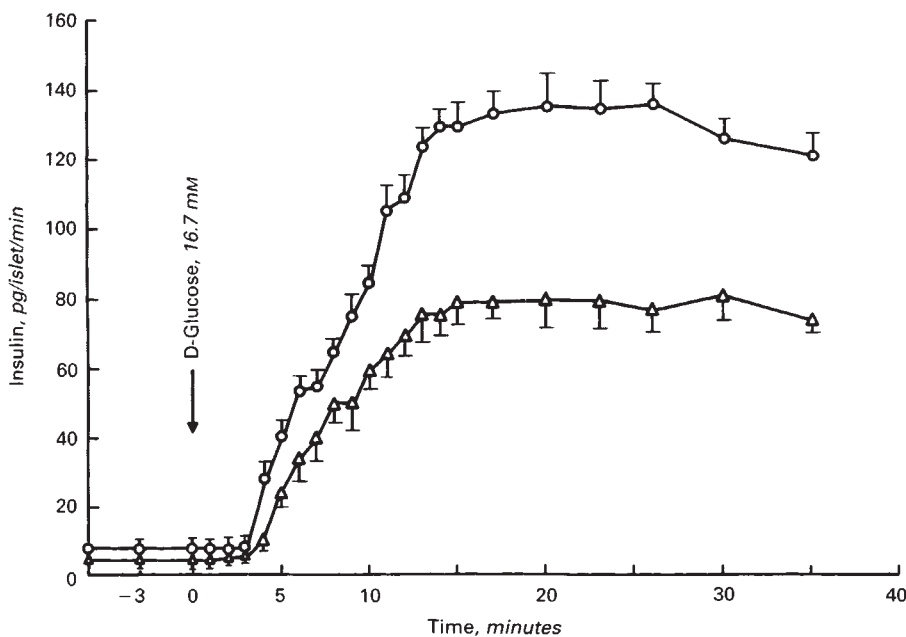Table 2 continued next page.

Fig. 4. *Dynamic insulin release from perifused pancreatic islets in 4 control animal (○) and 6 PTH-treated rats (△).* Each data point represents the mean value and brackets 1 SE.

PTX rats. The CRF-control animals demonstrated glucose intolerance with the area under the curve being significantly greater ($P < 0.05$) than in control rats. In contrast, glucose tolerance was preserved in CRF-PTX rats with an area under the curve not different from normal. Also blood glucose concentration at 45 and 60 minutes in CRF-control rats was significantly higher ($P < 0.01$) than corresponding values in both normal and CRF-PTX rats.

Insulin release by pancreatic islets

The biochemical data, insulin release during static studies, and calcium content of pancreas in control, CRF-control and CRF-PTX rats are given in Table 2 and Figure 2. There were no significant differences in the body weight and in the blood levels of calcium and phosphorus among the three groups. The 5/6 nephrectomy resulted in a significant rise in the blood concentrations of creatinine and magnesium, but the latter two param-

eters were not significantly different among the two CRF groups of animals. Calcium content of pancreas in CRF-control (11.2 ± 0.7 g/kg dry wt) was significantly higher ($P < 0.01$) than in normal (5.9 ± 0.9 g/kg dry wt) or CRF-PTX rats (6.7 ± 0.7 g/kg dry wt). The calcium content of the latter animals were not different from that of normal rats.

Insulin release induced by 16.7 mM glucose during static studies in CRF-control rats (54 ± 4 pg/islet/min) was significantly lower ($P < 0.01$) than that in normal (88 ± 4 pg/islet/min) or in CRF-PTX rats (90 ± 10 pg/islet/min), and insulin release in the latter two groups were not significantly different. In presence of 16.7 mM glucose, both IBMX and forskolin significantly ($P < 0.01$) stimulated insulin release in all three groups of animals, but insulin release was significantly lower in CRF-control than in normal and CRF-PTX. However, the increments in insulin release induced by IBMX and forskolin were not different among the three groups of animals.

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Table 2. *Continued*

Insulin release pg/islet/min				Forskolin 16.7 mM glucose	Δ2	Total pancreatic calcium[a] g/kg dry wt
2.8 mM glucose	16.7 mM glucose	IBMX 16.7 mM glucose	Δ1			
5.6 ± 0.7	86 ± 9	148 ± 14	62 ± 12	131 ± 12	45 ± 7	5.9 ± 0.9
3.3 ± 0.5	56 ± 5	93 ± 6	37 ± 13	87 ± 7	31 ± 9	11.2 ± 0.7
6.2 ± 1.6	91 ± 9	141 ± 12	50 ± 11	142 ± 9	51 ± 15	6.4 ± 0.7
NS	<0.01	<0.01	NS	0.01	NS	<0.01
NS	NS	NS	NS	NS	NS	NS
NS	<0.01	<0.01	NS	0.01	NS	<0.01

Insulin release during the dynamic studies is shown in Figure 3. It is evident that insulin release in CRF-control rats was lower than in control or CRF-PTX animals. Early insulin release (5 min) calculated from area under the curve in CRF-control rats (59 ± 31 pg/islet) was significantly lower ($P < 0.01$) than in control (209 ± 20 pg/islet) or CRF-PTX (187 ± 26 pg/islet). Total insulin release (31 min) was also significantly lower ($P < 0.01$) in the CRF-control (1082 ± 104 pg/islet) than in control (3196 ± 216 pg/islet) or in CRF-PTX (2856 ± 356 pg/islet) rats. There were no significant differences between early or total insulin release between control and CRF-PTX rats.

The long term administration of 1-84 PTH to rats did not cause significant changes in their weight (355 ± 15 vs. 360 ± 17 g), blood creatinine (0.7 ± 0.09 vs. 0.6 ± 0.10 mg/dl), blood calcium (9.7 ± 0.5 vs. 9.1 ± 0.4 mg/dl) or blood magnesium (1.87 ± 0.4 vs. 1.71 ± 0.1 mg/dl). The blood levels of phosphorus decreased modestly but significantly after PTH administration (6.7 ± 0.3 vs. 7.6 ± 0.3 mg/dl, $P < 0.01$). The calcium content of the pancreas was significantly higher ($P < 0.05$) in PTH-treated animals (7.4 ± 0.6 g/kg dry wt) as compared to control rats (5.9 ± 0.9 g/kg dry wt).

Figure 4 depicts insulin release during dynamic studies in four control rats and five PTH-treated rats. The initial insulin release (5 min) in the PTH-treated animals (140 ± 12 pg/islet) was significantly lower ($P < 0.05$) than in control (208 ± 28 pg/islet). Also total insulin release (31 min) in PTH-treated rats (1979 ± 82 pg/islet) was significantly lower ($P < 0.01$) than control rats (3220 ± 82 pg/islet).

Discussion

The results of the present study show that rats with six weeks of CRF developed glucose intolerance, and this abnormality was reversed by prior parathyroidectomy. These data are similar to those reported in dogs [1] and in humans [2, 3] and indicate that this rat model is suitable for the study of the effect of CRF and PTH on insulin release.

Available data indicate that the state of glucose intolerance in uremia is due to tissue resistance to insulin combined with impaired ability of the β-cell to release appropriate amounts of insulin [5, 10] to overcome the peripheral resistance to the hormone. Studies in humans [2, 3] and dogs [1] with chronic renal failure have demonstrated that the state of secondary hyperparathyroidism may impair insulin release from the pancreas. Indeed, correction of the hyperparathyroidism either by medical suppression of the parathyroid gland activity or by parathyroidectomy [1–4] was associated with an increase in insulin release and correction of the carbohydrate intolerance without a change in the peripheral resistance to insulin action. Our data provide direct evidence that both the early and total insulin release during stimulation with glucose, by islets from rats with six weeks of CRF and intact parathyroid gland are significantly impaired.

The blood levels of PTH were not measured in our animals since the assay of PTH in the rat is not widely available and is done in very few laboratories. However, current data indicate that chronic renal failure in humans [11–13] and in dogs [1] is associated with secondary hyperparathyroidism, and increased activity of the parathyroid glands develops in rats within hours after the induction of renal failure [14]. We can, therefore, assume with high degree of confidence that our rats did have secondary hyperparathyroidism.

Thus, the defect in insulin release by pancreatic islets obtained from CRF-control rats appears to be due to the state of secondary hyperparathyroidism of CRF. Two observations in our study support this conclusion. First, both early and total insulin release from islets of normocalcemic parathyroidectomized rats with similar degree and duration of CRF were not different from results obtained in normal rats. Second, insulin release by islets from rats with normal renal function and a state of hyperparathyroidism produced by PTH administration for six weeks was also significantly lower than insulin release from islets of normal rats. Thus, insulin release is impaired in the presence of hyperparathyroidism independent of the presence or absence of CRF.

Available data indicate that stimulation of insulin release by glucose is mediated by a rise in cytosolic calcium concentration [15, 16]. It is believed that the increase in cytosolic calcium concentration during the early phase of insulin release is brought about by mobilization of calcium from intracellular stores [16, 17] and this process may require energy [18]. On the other hand, the rise in cytosolic calcium during the second

phase of insulin release is due to an increase in calcium influx into the β-cells [15, 19, 20]. Many other agents that act as insulin secretogogues such as amino acids or acetylcholine also increase cytosolic calcium [20, 21].

The mechanism through which excess PTH may blunt glucose-induced insulin release is not as yet delineated. Several possibilities should be considered. PTH has been shown to acutely stimulate calcium influx into many cells [22–27], and one would, therefore, expect that this hormone should stimulate rather than blunt insulin release. However, it is possible that the effect of a chronic excess of PTH on the β-cells is different from an acute effect. A corollary to a different effect of acute or chronic excess of PTH is found in observations in other systems. Excess amount of the hormone acutely stimulates chronotropic [28] and inotropic [29] properties of the heart cells and enhances random motility of polymorphonuclear leucocytes [30], but chronic exposure to excess PTH decreases or stops the beating of heart cells [28], impairs the metabolism and function of the heart [31] and reduces random motility of the leukocytes [30]. The available data on insulin response to hyperglycemia with primary hyperparathyroidism (a state of chronic excess of PTH) do not help resolve these issues in that these patients have hypercalcemia and/or hypophosphatemia, both of which affects insulin release.

It is possible that the effects of changes in cytosolic calcium of the β-cells on insulin release depends on the magnitude of the change and its duration. One may speculate that chronic exposure to PTH may lead to sustained rise in cytosolic calcium. Direct evidence for such a notion is not available, but the calcium content of the pancreas was significantly increased in the rats with hyperparathyroidism with and without CRF. If this change in calcium content is equally distributed in the pancreas, one may assume that calcium overloading of the islet cells is present. Under such circumstances the capacity of cellular oganelles to sequester calcium may approach saturation and a new steady state with higher cytosolic calcium may develop. This proposition requires confirmation with direct measurements of cytosolic calcium.

Frankel, Atwater and Grodsky [32] have shown that higher cytosolic calcium may activate potassium permeability, cause repolarization of cell membrane and blunt insulin release. Also, it is known that glucose-induced insulin release plateaus as cytosolic calcium reaches a certain level [15]. If the β-cells during states of chronic hyperparathyroidism have high concentration of calcium than normal, the critical level of cytosolic calcium at which glucose-induced insulin secretion plateaus would be reached earlier and insulin release would be blunted.

Chronic exposure to PTH in rats caused impairment in energy production, shuttle and utilization by the myocardium [31] and skeletal muscle [33]. It is, therefore, possible that a similar effect on β-cells of the pancreatic islet may reduce energy availability necessary for calcium mobilization during the initial phase of insulin release. Such an effect may blunt or abolish the glucose-induced early insulin release and provide an explanation for the observed reduction in insulin release in CRF-control and PTH-treated rats.

It also appears that the reduction in insulin release in our rats with excess PTH does not seem to be related to derangement in the adenylate cyclase-cyclic AMP system. Indeed, an increase in islet cyclic-AMP produced either by forskolin, which stimu-

lates adenylate cyclase activity [34], or by IBMX, which inhibits phosphodiesterase activity [15], caused a similar increments in insulin secretion in the normal rats and in CRF animal with and without excess PTH.

Finally, a potential role for an alteration of $1,25(OH)_2D$ metabolism in the genesis of the reduced insulin release in CRF-control and PTH-treated animals should be considered. It has been demonstrated that the chick pancreas contains receptors for $1,25(OH)_2D$ [35], and a vitamin D calcium-binding protein is present in the pancreas and β-cells of the chick and rat [36–38]. Furthermore, Kadowaki and Norman [39] showed that vitamin D deficiency in the rat is associated with a reduction in glucose-induced insulin release from the pancreas, and $1,25(OH)_2D_3$ restored this abnormality. Thus, it appears that this vitamin D metabolite may stimulate insulin release. In our studies, the CRF-PTX rats should have lower levels of $1,25(OH)_2D$ than CRF-control animals and the PTH-treated rats should have elevated levels of this metabolite, since PTH stimulates production of $1,25(OH)_2D$ [40, 41]. However, the insulin release was higher in PTX-CRF rats than CRF-control rats and lower in PTH-treated animals than control ones. Thus, these findings do not support the notion that the reduced insulin release noted in our study is due to a deficiency in $1,25(OH)_2D$.

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