Reduced Activity of Na\textsuperscript{+}-K\textsuperscript{+} ATPase of Pancreatic Islets in Chronic Renal Failure: Role of Secondary Hyperparathyroidism\textsuperscript{1}

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ABSTRACT
The activity of Na\textsuperscript{+}-K\textsuperscript{+} ATPase of pancreatic islets modulates their insulin secretion. The study presented here examined the activity of this enzyme in pancreatic islets of chronic renal failure (CRF) rats in an effort to further delineate the mechanisms of impaired insulin secretion in CRF. The V\textsubscript{max} of Na\textsuperscript{+}-K\textsuperscript{+} ATPase, but not its K\textsubscript{m}, and the AlP content are significantly reduced in islets of CRF rats that have elevated levels of parathyroid hormone (PTH). These derangements are prevented by prior parathyroidectomy of CRF rats (low blood levels of PTH) or by their treatment with the calcium channel blocker verapamil; these latter rats have sustained elevation of blood levels of PTH. The data indicate that the chronic excess blood levels of PTH in CRF initiates events (augmented entry of calcium) that lead to the reduction in ATP content and in V\textsubscript{max} of Na\textsuperscript{+}-K\textsuperscript{+} ATPase of pancreatic islets. Reducing the blood levels of PTH by parathyroidectomy or blocking the action of PTH on calcium entry into cells by verapamil prevents these derangements. The results suggest that chronic inhibition of Na\textsuperscript{+}-K\textsuperscript{+} ATPase may participate in the processes underlying the impaired insulin secretion in CRF.

Key Words: Insulin, PTH, Na\textsuperscript{+}-K\textsuperscript{+} ATPase

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Available data indicate that Na\textsuperscript{+}-K\textsuperscript{+} ATPase activity of pancreatic islet membranes modulates their insulin release after acute exposure to a secretagogue (1-3). Levin et al. (3) reported that the exposure of pancreatic islets to glucose was associated with suppression of their Na\textsuperscript{+}-K\textsuperscript{+} ATPase activity. This observation suggests that inhibition of the activity of islet Na\textsuperscript{+}-K\textsuperscript{+} ATPase could result in insulin secretion. Indeed, ouabain, an inhibitor of Na\textsuperscript{+}-K\textsuperscript{+} ATPase (4), augmented insulin secretion by pancreatic islets (1). On the other hand, diphenylhydantoin, an enhancer of Na\textsuperscript{+}-K\textsuperscript{+} ATPase (5), reduced insulin secretion.

Chronic renal failure (CRF) impairs glucose-induced insulin secretion by pancreatic islets (6). If Na\textsuperscript{+}-K\textsuperscript{+} ATPase of pancreatic islets participates in the genesis of impaired insulin secretion in CRF, one may speculate that the activity of this enzyme is enhanced in CRF. However, CRF is known to cause inhibition of Na\textsuperscript{+}-K\textsuperscript{+} ATPase activity in a sundry of cells, including red blood cells (7,8), leukocytes (9), skeletal muscle (10), cardiac sarcomere (11,12), and brain synaptosomes (13), and it is possible that a similar effect occurs in pancreatic islets. Data on the activity of Na\textsuperscript{+}-K\textsuperscript{+} ATPase of pancreatic islets in CRF are lacking.

The study presented here examined the effect of CRF on Na\textsuperscript{+}-K\textsuperscript{+} ATPase of pancreatic islet membranes and whether a chronic change in its activity participates in the genesis of the impaired insulin secretion in CRF.

METHODS
Male Sprague-Dawley rats weighing 225 to 325 g were studied. They were fed normal rat chow diet (ICN Nutritional Biochemical, Cleveland, OH) throughout the study and were allowed to drink ad libitum. Experiments were performed in five groups of animals: (1) normal rats; (2) rats with CRF of 42 days duration; (3) normocalcemic parathyroidectomized CRF rats (CRF-PTX); (4) CRF rats treated with verapamil (0.1 \( \mu \)g/g body wt), which was given s.c. twice a day from day 1 of CRF (CRF-V); and (5) normal rats treated with verapamil for 42 days (normal-V). CRF was produced by 2/3 nephrectomy; the animals underwent right 2/3 nephrectomy through a flank incision and a week later, a left nephrectomy was performed. PTX was performed by electroc
tery, 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 drink water containing 5% calcium gluconate. This procedure is adequate to normalize serum calcium in the PTX rats. Seven days after PTX, the rats were subjected to % nephrectomy. Two days before the sacrifice of the animals, they were housed in metabolic cages for the measurement of creatinine clearance. The animals were killed by decapitation 42 days after completion of the % nephrectomy in CRF rats (CRF, CRF-PTX, CRF-V) and 42 days after verapamil treatment in normal rats. The pancreas was removed and was dissected free of adipose tissue and lymph nodes. The islets of Langerhans were isolated by the collagenase digestion method (14) and were picked under a dissecting microscope.

ATP Content of Islets

The ATP content of the islets was measured by the methods of Ashcroft et al. and Lundin et al. (16). The islets were placed in 400 μL of Krebs-Ringer-bicarbonate (KRBHA) incubation medium containing 2.8 mM D-glucose and were incubated for 30 min. At the end of the incubation, 200 μL of the medium containing the islets was mixed with trichloroacetic acid (TCA) with a final concentration of 2.5%. The tube containing the mixture was immersed in liquid nitrogen and was stored at -70°C. On the day of the assay, the mixture was thawed and neutralized with 2 N KHCO₃. Samples of 50 μL of the mixture were assayed for ATP. The ATP measurements were made by the firefly luminescence assay with the LAD 535 Lumimeter (Turner Design, Sunnyvale, CA). ATP standards were prepared with KRBHA medium and contained the same amount of TCA and bicarbonate as did the islet extracts.

Measurements of Na⁺-K⁺ ATPase

For the measurement of Na⁺-K⁺ ATPase activity, islet membranes were prepared by the method of Tung et al. (17). Two hundred islets were suspended in 2 mL of 300 mM sucrose and 10 mM Tris-HCl (pH 7.4) at 4°C and were sonicated with a Brausonic 1510 sonicator (B-Braun Instruments, Melsungen, AG, Germany) while on ice for 10 s. The suspension was centrifuged at 35,000 × g for 35 min at 4°C in a Beckman ultracentrifuge (Model L5-55D; Beckman Instruments, Palo Alto, CA) with a Ti-50 rotor. The pellet was suspended in 1 mM Tris-EDTA (pH 7.4) and was divided into several samples for subsequent measurement of enzyme activity and protein. One hundred microliters of membrane homogenate was added to 850 μL of media with similar composition except that NaCl and KCl were equimolarly replaced by Tris-HCl and 0.1 mM of ouabain was added. Both samples were preincubated for 10 min at 37°C; thereafter, 50 μL of 4 to 40 mM stock ATP was added, bringing the final incubation volume to 1 mL and the ATP concentration to 0.2, 0.6, 0.8, 1.0, 1.5 and 2 mM. The ATP level was measured by the firefly luminescence technique (6) before and at the end of a 15-min incubation at 37°C. The values of Na⁺-K⁺ ATPase were calculated as the difference between the ATP levels consumed by islet membranes during this 15-min incubation in the presence and absence of NaCl and KCl. The activity of the enzyme is expressed as micromoles of ATP consumed per milligram of protein per hour. Our preliminary studies on islet membranes of normal rats showed that the relationship between the Na⁺-K⁺ ATPase activity and incubation time was linear during the first 45 min of incubation; thus, a 15-min incubation was subsequently used for the study of enzyme activity in the membranes of experimental animals.

The measurements of calcium and magnesium concentrations in plasma were made by a Perkin-Elmer atomic absorption spectrophotometer (Model 503; Perkin-Elmer Corp., Norwalk, CT) and those of creatinine and phosphorus were made by a Technicon Autoanalyzer (Technicon Instrument Inc., Tarrytown, NY). The serum level of parathyroid hormone (PTH) was determined by an INS-PTH immunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay recognizes the amino-terminal fragment of PTH. The lowest detectable level is 3 pg/mL, the interassay variation is 7.3%, and the intra-assay variation is 4%. Statistical analysis was done with one-way analysis of variance and Tukey's honest statistical difference (HSD) test for multiple comparison between groups and unpaired t test for comparison of parameters within each group. Data are expressed as mean ± SE.

RESULTS

The body weight and biochemical data in the five groups of animals studied are given in Table 1. The % nephrectomy was associated with significant (P < 0.01) elevation in the concentrations of creatinine in plasma, with the values being three to four times higher than normal. The body weight and plasma concentrations of calcium in the five groups were not different. Plasma phosphorus in CRF was modestly but significantly (P < 0.05) lower than that in the other four groups of animals. The serum levels of PTH in CRF and CRF-V rats were significantly (P < 0.01) higher than those in normal, normal-V, and CRF-PTX rats, and the values in the latter animals were significantly (P < 0.01) lower than those in the other four groups.
TABLE 1. Body weight, biochemical variables, and serum PTH in the five groups of rats at the time of sacrifice

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Plasma (mg/dL)</th>
<th>Serum PTH (pg/mL)</th>
<th>Creatinine Clearance (µL/min/100 g)</th>
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<tr>
<td></td>
<td></td>
<td>Cr</td>
<td>Ca</td>
<td>P</td>
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<tr>
<td>Normal (N = 11)</td>
<td>296 ± 3.8</td>
<td>0.3</td>
<td>0.04</td>
<td>9.2</td>
</tr>
<tr>
<td>CRF (N = 8)</td>
<td>281 ± 15.6</td>
<td>1.1</td>
<td>0.14</td>
<td>8.8</td>
</tr>
<tr>
<td>CRF-PTX (N = 12)</td>
<td>293 ± 14.7</td>
<td>1.0</td>
<td>0.02</td>
<td>9.1</td>
</tr>
<tr>
<td>Normal-V (N = 9)</td>
<td>295 ± 5.1</td>
<td>0.3</td>
<td>0.04</td>
<td>8.7</td>
</tr>
<tr>
<td>CRF-V (N = 8)</td>
<td>248 ± 11.7</td>
<td>1.3</td>
<td>0.13</td>
<td>9.0</td>
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* P < 0.01 versus normal and normal-V.
* P < 0.05 versus normal.
* P < 0.01 versus normal, normal-V, and CRF-PTX.
* P < 0.01 versus all groups.

** Figure 1.** ATP content of pancreatic islets. Each datum point represents one animal. Brackets denote mean ± 1 SE.

** Figure 2.** A representative study of the $K_m$ of Na$^+$-K$^+$ ATPase of pancreatic islet membrane. The data are presented as the Lineweaver-Burk plot demonstrating linearity. The plot represents the reciprocal of the initial concentrations of the substrate(s), which yields a line whose slope is $K_m/V_{max}$, whose y-intercept is $1/V_{max}$, and whose x intercept is $-1/K_m$.

** Figure 3.** $V_{max}$ of Na$^+$-K$^+$ ATPase of pancreatic islets. Each datum point represents one animal. Brackets denote mean ± 1 SE.
DISCUSSION

The data of the study presented here demonstrate that the $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase of pancreatic islet membranes from CRF rats is significantly reduced. This derangement was prevented by prior PTX of the CRF rats or by their treatment with the calcium channel blocker verapamil. This decrease in the $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase could be due to a decrease in the $V_{\text{max}}$ of each enzyme unit and/or a decrease in the number of pump units per cell; our data do not differentiate between these two possibilities.

Our results are consistent with other observations demonstrating that CRF is associated with inhibition of Na$^+$/K$^+$ ATPase of other cells (7–10) and shed further light on the potential mechanisms involved in this derangements. The observation in our study that PTX of CRF rats prevented the decrease in the $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase indicates that either the elevated blood levels of PTH or a metabolic consequence of the chronic excess of PTH is responsible for this abnormality.

PTH is known to augment the entry of calcium into many cells (18-22), and chronic excess of PTH in the presence (6,13,23-25) or absence of CRF (13,26) is associated with a sustained elevation in the resting levels of cytosolic calcium ([Ca$^{2+}$]$i$]. Indeed, pancreatic islets of CRF rats do have elevated levels of resting [Ca$^{2+}$]$i$, and this rise in [Ca$^{2+}$]$i$ is prevented by PTX or by treatment with verapamil (6,27). Our data are consistent with the proposition that the PTH-induced rise in [Ca$^{2+}$]$i$ of islets and not PTH, per se, is responsible for the reduction in the $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase, because the activity of this enzyme is not reduced in islets of CRF-V rats despite significant elevation in their blood levels of PTH. Support of this proposition is provided by observations in another experimental model. Indeed, chronic phosphate depletion in the rat is associated with sustained elevation in [Ca$^{2+}$]$i$ and reduced $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase of their islets (28); furthermore, normalization of the [Ca$^{2+}$]$i$ by verapamil treatment of the phosphate-depleted rats was associated with correction of reduced Na$^+$/K$^+$ ATPase of the islets (28).

The sustained elevation in [Ca$^{2+}$]$i$ is associated with a fall in ATP content of cells (6,13) because the increase in cellular calcium burden inhibits mitochondrial oxidation and ATP production (26,29–31). Our data, indeed, demonstrate that the islets of CRF rats display significant elevation in the basal level of [Ca$^{2+}$]$i$ and have significantly reduced ATP content (6). This decrease in the ATP content of the islets could be responsible, at least in part, for the reduction in the $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase. Indeed, Niki et al. (32) reported that the activity of Na$^+$/K$^+$ ATPase depends on the ATP concentration in HIT-T15 beta cells. The activity of the enzyme decreased progressively as ATP was lowered from 3 to 1.5 mM. Although the affinity of the enzyme for ATP is in the micromolar range, its activity is altered by a millimolar change in ATP concentration, indicating that ATP is compartmentalized within the cell with its concentration in the submembrane pool being considerably lower than that in the cytosolic pool.

Certain data suggest that vanadium (33) and digitals-like compounds are present in the blood of CRF patients (34), and such substances could participate in the reduction of the activity of Na$^+$/K$^+$ ATPase of the pancreatic islets. If this process indeed operates in our rats with CRF, one must accept that PTX of CRF rats or treatment of these animals with verapamil prevents the accumulation or interferes with the action of such compounds; evidence for this latter suggestion is not available.

Taken together, our data permit the formulation of the proposition that chronic excess of PTH in CRF enhances the entry of calcium into pancreatic islets and causes a sustained elevation in their [Ca$^{2+}$]$i$. The latter inhibits mitochondrial oxidation and ATP production, resulting in a consequent fall in ATP content, which, in turn, leads to a reduction in the $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase. Prevention of the augmented entry of calcium into the islets of CRF rats either by reduction of blood levels of PTH (CRF-PTX rats) or by the blocking of the PTH-induced calcium entry into islets (CRF-V rats) results in normalization of ATP content and $V_{\text{max}}$ of Na$^+$/K$^+$ ATPase of the islets.

This phenomenon is not unique to the pancreatic islets. CRF is also associated with a rise in [Ca$^{2+}$]$i$, a reduction in ATP content, and an inhibition of Na$^+$/K$^+$ ATPase activity of brain synaptosomes (13). All of these derangements are prevented by PTX or by the treatment of CRF rats with verapamil (13,35).

Because acute inhibition of islet Na$^+$/K$^+$ ATPase is associated with stimulation of insulin secretion (1–3), one would expect that insulin secretion by pancreatic islets in CRF should be stimulated because Na$^+$/K$^+$ ATPase activity is reduced. However, insulin secretion in CRF is impaired (6,36). It is possible, therefore, that the effects of acute or chronic inhibition of Na$^+$/K$^+$ ATPase on insulin secretion are different. Alternatively, the islets in CRF display significant elevation in [Ca$^{2+}$]$i$, reduction in ATP, and impairment in their glucose metabolism (6). All of these changes conspire to inhibit insulin secretion and may, therefore, mask any stimulatory effect secondary to inhibition of Na$^+$/K$^+$ ATPase activity. We would like to emphasize, however, that the proposed interaction between chronic inhibition of pancreatic islet Na$^+$/K$^+$ ATPase and the islet's insulin secretion in CRF is speculative and requires further documentation.

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