Chronology of Cellular Events Leading to Derangements in Function of Pancreatic Islets in Chronic Renal Failure

Edi Levi, George Z. Fadda, Prasert Thanakitcharu, and Shaul G. Massry

ABSTRACT
In chronic renal failure (CRF), a multitude of metabolic derangements occur in the pancreatic islets, resulting in impaired glucose-induced insulin secretion. These abnormalities include a rise in the basal level of cytosolic calcium ([Ca\textsuperscript{2+}]i) in the islet, a decrease in their basal and stimulated ATP and ATP/ADP ratio, a reduction in the V\textsubscript{max} of Ca\textsuperscript{2+}ATPase and Na\textsuperscript{+}-K\textsuperscript{+}ATPase, and an impaired glucose-induced calcium signal. The sequence of events that lead to these derangements and to the impairment in insulin secretion during the evolution of CRF are not defined. The study presented here examined this issue by measuring the metabolic profile of pancreatic islets weekly during the evolution of CRF over a period of 6 wk. The results show that serum levels of parathyroid hormone (PTH) begin to rise during the first week of CRF. The V\textsubscript{max} of Ca\textsuperscript{2+}ATPase and Na\textsuperscript{+}-K\textsuperscript{+}ATPase increased during weeks 1 to 3 of CRF but fell to low levels thereafter. At week 3 of CRF, the basal level of (Ca\textsuperscript{2+})i began to rise, whereas basal and the stimulated ATP content and ATP/ADP ratio started to fall. Glucose-induced calcium signal, (Δ(Ca\textsuperscript{2+})i)/basal (Ca\textsuperscript{2+})i, and insulin secretion became abnormally low between weeks 3 and 6 of CRF. The data allow the following formulation: as serum levels of PTH begins to rise, calcium entry into islets is augmented; this in turn will stimulate the activity of Ca\textsuperscript{2+}ATPase and the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger, and hence, calcium extrusion out of the islets is increased. As a result, (Ca\textsuperscript{2+})i remains normal during the first 2 wk of CRF. An activation of Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger may result in accumulation of sodium into islet, an event that would activate the Na\textsuperscript{+}-K\textsuperscript{+}ATPase. As calcium entry is further augmented by the progressive rise in serum PTH levels, mitochondrial oxidation and ATP production would be reduced, resulting in lower ATP content. This fall in ATP causes a reduction in the V\textsubscript{max} of Ca\textsuperscript{2+}ATPase and Na\textsuperscript{+}-K\textsuperscript{+}ATPase, and therefore, calcium extrusion out of the islets is reduced; consequently, (Ca\textsuperscript{2+})i rises. With the falling ATP content and the rise in (Ca\textsuperscript{2+})i, glucose-induced insulin secretion is impaired because of alterations in the closure of ATP-dependent potassium channels and reduction in the glucose-induced calcium signal (Δ(Ca\textsuperscript{2+})i) and/or (Δ(Ca\textsuperscript{2+})i)/basal (Ca\textsuperscript{2+})i.

Key Words: Parathyroid hormone, insulin, Ca\textsuperscript{2+} ATPase, Na\textsuperscript{+}-K\textsuperscript{+} ATPase, cytosolic calcium, ATP

Available data indicate that chronic renal failure (CRF) is a state of increased calcium burden of cells with increased basal levels of cytosolic calcium ([Ca\textsuperscript{2+}]i) [1-7]. This derangement has been attributed [1,3,5] to the elevated blood levels of parathyroid hormone (PTH) in CRF [3,8-10], because PTH is known to augment the entry of calcium into cells (11,12). However, an increase in calcium entry into cells should not result in elevation in [Ca\textsuperscript{2+}]i unless the processes responsible for calcium removal from cytosol are also impaired. Indeed, we have found that the V\textsubscript{max} values of Ca\textsuperscript{2+}ATPase and Na\textsuperscript{+}-K\textsuperscript{+}ATPase, enzymes that are involved directly or indirectly in calcium extrusion out of cells (13,14), are reduced (3,4,15,16).

Data obtained from several studies carried out on pancreatic islets (3,6,12,16) and brain synaptosomes (4,15) from rats with CRF allow the following formulation for the genesis of the rise in their [Ca\textsuperscript{2+}]i: as CRF progresses, PTH begins to rise and this would augment calcium entry into cells and increase their calcium burden, an event that would inhibit mitochondrial oxidation and ATP production with consequent fall in ATP content (17-19). The fall in ATP would result in impairment in the activity of Ca\textsuperscript{2+}ATPase and Na\textsuperscript{+}-K\textsuperscript{+}ATPase because ATP is required for the functional integrity of these enzymes (13,14,20). The reduction of the activity of these enzymes impairs calcium extrusion out of the cells. Thus, a vicious circle develops until a new steady state ensues with high [Ca\textsuperscript{2+}]i, low ATP content, and impaired activity of the calcium pumps. Indeed, pan-
Cycloheximide islets (3,6,12,16) and brain synaptosomes (4,15) from rats with stable CRF display these derangements.

This formulation implies a sequence of events that occurs over time during the progression of CRF. To test this hypothesis, the study presented here examined the chronology of the changes in blood levels of PTH. [Ca\(^{2+}\)], basal and stimulated ATP content, and V\(_{\text{max}}\) of Ca\(^{2+}\)-ATPase and Na\(^+-K^+\)-ATPase of pancreatic islets weekly over 6 wk of CRF. As a measure of the effects of the changes in these parameters on the function of islets, we simultaneously measured glucose-induced insulin secretion.

METHODS

Studies were performed on 374 male Sprague-Dawley rats weighing 275 to 300 g. They were fed normal rat chow diet (Wayne Research Animals Diets, Chicago, IL) throughout the study and were allowed to drink ad libitum. CRF was produced by two-stage 5% nephrectomy. 317 rats underwent right partial nephrectomy. 317 rats underwent right partial nephrectomy through a flank incision: a week later, a left nephrectomy was performed. The animals were studied at 7, 14, 21, 28, 35, and 42 days after the left nephrectomy. A group of 57 normal rats served as controls.

Two days before being euthanized, the rats were housed in metabolic cages for the collection of two consecutive 24-h urine outputs. On the designated day, the animals were decapitated and the pancreas was dissected free of adipose tissue. Islets of Langerhans were isolated by the collagenase digestion method of Lacy and Kostianovsky (21) and picked free of exocrine tissue under a dissecting microscope.

Insulin secretion from 20 size-matched islets were evaluated under dynamic conditions by methods previously reported (3). In these dynamic studies of insulin release, the changes from baseline with time were examined by calculating the area under the curve for each study, allowing us to estimate insulin release during the initial phase and the total insulin release.

[Ca\(^{2+}\)] of the pancreatic islets was measured with Fura2-AM (Sigma Chemical Co., St. Louis, MO) as described by Komatsu et al. (22) and reported by us as well (23,24). This technique uses entire islets. Measurement of the Fura2-AM fluorescence was done with a Perkin Elmer fluorescence spectrophotometer (Model LS-5B; Perkin Elmer, Norwalk, CT) at an excitation wavelength of 340 nm and an emission wavelength of 510 nm. To eliminate the effect of autofluorescence due to cuvette, medium, and un-loaded islets, autofluorescence was measured before each experiment and was accounted for by setting the fluorometer to autozero before each measurement. Calculation of cytosolic calcium was made with the Grynkiewicz equation: [Ca\(^{2+}\)] = K\(_d\) (F - F\(_{\text{min}}\))/ (F\(_{\text{max}}\) - F) (25). Basal fluorescence was recorded for a period of 2 min, after which the glucose concentration in the cuvette was increased to 16.7 mM and the fluorescence was continuously monitored for 10 min. Maximal fluorescence (F\(_{\text{max}}\)) and minimal fluorescence (F\(_{\text{min}}\)) were estimated as previously reported (3).

Both ATP and ADP content of islets were measured simultaneously by the methods of Ashcroft et al. (26) and of Lundin et al. (27) as modified and reported by us (3,24). Samples of islet extract were assayed for ATP and ADP from rats with stable CRF display these derangements. As a measure of the AP content, the ATP measurements were made by the firefly luminescence assay with the LAD 535 Luminometer (Turner Design, Sunnyvale, CA).

Ca\(^{2+}\)-ATPase activity was measured in pancreatic islet membranes, which were prepared by the method described by Levin et al. (28) and modified by our laboratory (3). The protein concentration of the islets was estimated by the method of Bradford (29). The activity of Na\(^+-K^+\)-ATPase was measured in islet membranes prepared by the method of Tung et al. (30). The details of the methodology were reported by us (16,17).

The measurements of calcium concentrations in plasma were made by a Perkin-Elmer atomic absorption spectrophotometer (Model 503; Perkin Elmer Corp.) and those of creatinine and phosphorus were made by Technicon autoanalyzer (Technicon Instrument Inc., Tarrytown, NY). Serum level of 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 intraassay variation is 4%.

The area under the curve for insulin secretion by pancreatic islets from each experiment was estimated by the trapezoidal rule. Statistical analysis was done by one-way analysis of variance and Tukey's Honest Significant Difference test for multiple comparison between groups. Data are expressed as mean ± SE.

RESULTS

The body weight, biochemical data, and creatinine clearance of the seven groups of rats are given in Table 1. There were no significant differences in the body weight among the groups. This is consistent with our observations that rats with this degree of CRF do not gain weight within a 6-wk interval.
Plasma levels of creatinine increased and creatinine clearance decreased significantly after the first week of CRF and continued to increase and decrease, respectively, over the next 5 wk. The serum levels of PTH rose modestly but significantly during the first week of CRF and continued to rise with the values being 2.5 times that of normal at the end of the 6 wk of CRF.

Figure 1 depicts the data of the dynamic studies of glucose-induced insulin release from pancreatic islets obtained from normal rats as compared with those obtained from rats with CRF of 1- to 6-wk duration. Insulin secretion in the initial phase (5 min), the second phase (25 min), and the total (30 min) calculated from the area under the curve is depicted in Table 2. There were no significant differences in insulin release from islets obtained from rats with 1 and 2 wk of CRF as compared with those from normals. Rats with 3 wk of CRF displayed a normal first phase of insulin secretion, but the second phase was significantly (P < 0.01) inhibited. Rats with 4, 5, and 6 wk of CRF showed significant (P < 0.01) inhibition of the first as well as the second phase of insulin secretion.

The resting level of [Ca\(^{2+}\)]\(_{i}\) in normal rats (82 ± 2.0 nM) is consistent with that from our previous observations (23) and that of Komatsu et al. (22). Rats with 1 and 2 wk of CRF maintained a normal level of basal [Ca\(^{2+}\)]\(_{i}\). The basal level of [Ca\(^{2+}\)]\(_{i}\) was, however, significantly (P < 0.05) higher after 3 wk of CRF and continued to rise to 135 ± 4.9 nM at the end of 6 wk of CRF (Figure 2). The basal levels of [Ca\(^{2+}\)]\(_{i}\) obtained in this study in islets of rats with 6 wk of CRF are similar to those previously reported (133 ± 4.4 nM) from our laboratory in rats with similar duration of CRF (3). D-Glucose caused an increase in the [Ca\(^{2+}\)]\(_{i}\) of the pancreatic islets isolated from all groups of animals; the magnitude of the rise of [Ca\(^{2+}\)]\(_{i}\) ([Δ[Ca\(^{2+}\)]\(_{i}\)]) is shown in Figure 3. The [Δ[Ca\(^{2+}\)]\(_{i}\)] in islets of normal rats (171 ± 7.7 nM) was not significantly different from that observed in islets of rats with 1 (177 ± 0.9 nM), 2 (173 ± 16.9 nM), 3 (168 ± 8.8 nM), and 4 (162 ± 15.3 nM) wk of CRF. At the fifth and sixth weeks of CRF, the glucose-induced rise in [Ca\(^{2+}\)]\(_{i}\) was significantly (P < 0.05 to < 0.01) lower than normal (143 ± 6.4) and 126 ± 15 nM, respectively. The ratio of [Δ[Ca\(^{2+}\)]\(_{i}\)]/basal [Ca\(^{2+}\)]\(_{i}\) after exposure to glucose was significantly (P < 0.05 lower after 3 wk of CRF and continued to fall thereafter (P < 0.01; Figure 3).

Table 3 provides the simultaneous values of ATP and ADP content and of ATP/ADP ratio in islets from normal rats and rats with different durations of CRF. The basal level of ATP and the basal ATP/ADP ratio as well as the glucose-stimulated ATP and ATP/ADP ratio were significantly (P < 0.01) lower than normal at the end of the third week of CRF and continued to fall further thereafter. The ADP content in islets of rats with 4, 5, and 6 wk of CRF was lower (P < 0.01) than those in control.

Table 4 depicts the V\(_{\text{max}}\) of Ca\(^{2+}\)ATPase and Na\(^{+}\)-K\(^{+}\)ATPase of pancreatic islets in normal rats and the various groups of CRF rats. The V\(_{\text{max}}\) of Ca\(^{2+}\)ATPase after 1 and 2 wk of CRF was significantly (P < 0.01) higher than that in normal rats. The values then began to fall and were significantly (P < 0.01) lower than normal during the third through the sixth weeks of CRF. The V\(_{\text{max}}\) of Na\(^{+}\)-K\(^{+}\)ATPase activity began to rise after the induction of CRF, and the values were significantly higher after 2 (P < 0.05) and 3 (P < 0.01) wk of CRF. Thereafter, the V\(_{\text{max}}\) of this enzyme decreased, and values were significantly (P < 0.01) lower than normal after the fourth through sixth weeks of CRF.
Figure 1. Dynamic studies of glucose-induced insulin secretion by perfused pancreatic islets. Insulin secretion was first measured during dynamic perfusion with Krebs-Ringer solution containing 2.8 mM D-glucose. At zero time, the glucose concentration of the perfusate was increased to 16.7 mM. Each datum point represents the mean value, and the brackets denote 1 SE. W, week.
TABLE 2. Glucose-induced insulin release from islets of normal rats and from those of CRF animals

<table>
<thead>
<tr>
<th></th>
<th>Initial Phase, pg/islets·5 min</th>
<th>Late Phase, pg/islets·25 min</th>
<th>Total, pg/islets·30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal CRF</td>
<td>6</td>
<td>1803 ± 83</td>
<td>2265 ± 91</td>
</tr>
<tr>
<td>1st wk</td>
<td>6</td>
<td>1784 ± 38</td>
<td>2283 ± 49</td>
</tr>
<tr>
<td>2nd wk</td>
<td>8</td>
<td>1721 ± 53</td>
<td>2224 ± 71</td>
</tr>
<tr>
<td>3rd wk</td>
<td>8</td>
<td>1420 ± 98°</td>
<td>1862 ± 97°</td>
</tr>
<tr>
<td>4th wk</td>
<td>6</td>
<td>873 ± 80°</td>
<td>1168 ± 72°</td>
</tr>
<tr>
<td>5th wk</td>
<td>8</td>
<td>762 ± 77°</td>
<td>1031 ± 82°</td>
</tr>
<tr>
<td>6th wk</td>
<td>6</td>
<td>754 ± 94°</td>
<td>973 ± 111°</td>
</tr>
</tbody>
</table>

*P < .01 versus normal and first and second weeks of CRF.

Figure 2. Resting levels of [Ca2+]i of pancreatic islets. Each column represents mean values of studies from 8 to 12 rats, and brackets denote 1 SE. *P < 0.05 and **P < 0.01 versus other groups of rats.

The chronologic relationship between the various parameters studied during the evolution of CRF over a period of 6 wk is depicted in Figure 4.

DISCUSSION

The results of this study demonstrate that the various derangements in the metabolic profile of the pancreatic islets of CRF rats became evident at different times and followed a distinct sequence during the evolution of CRF.

The first two abnormalities were a rise in the blood levels of PTH and an increase in the Vmax of Ca2+ATPase. This observation is consistent with the proposition that the rise in the blood levels of PTH augments the entry of calcium into the islets, and such an event triggers the activation of the Ca2+ATPase in an effort to prevent calcium accumulation in the cytosol of the islets. Thus, it appears that the increase in the Vmax of Ca2+ATPase of the islets during the first and second weeks of CRF represents an adaptive response to the PTH-induced entry of calcium into the islets. As a consequence, the basal levels of [Ca2+]i of the islets remained normal during weeks 1 and 2 of CRF.

Although the activity of the Na+-Ca2+ exchanger was not determined in our study, it is reasonable to suggest that this system is also activated as a consequence of the PTH-induced calcium entry into the
TABLE 3. ATP and ADP content and ATP/ADP ratio in normal rats and rats with variable duration of CRF

<table>
<thead>
<tr>
<th>N</th>
<th>ATP pmole/islet</th>
<th>ADP pmol/islet</th>
<th>ATP/ADP Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8 mM α-Glucose</td>
<td>16.7 mM α-Glucose</td>
<td>2.8 mM α-Glucose</td>
<td>16.7 mM α-Glucose</td>
</tr>
<tr>
<td>Normal CRF (wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>14.5 ± 0.23</td>
<td>26.3 ± 0.40</td>
<td>3.5 ± 0.08</td>
</tr>
<tr>
<td>1</td>
<td>14.9 ± 0.15</td>
<td>25.7 ± 0.37</td>
<td>3.5 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>13.9 ± 0.16</td>
<td>25.5 ± 0.26</td>
<td>3.5 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>11.6 ± 0.31°</td>
<td>20.6 ± 0.35°</td>
<td>3.4 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>9.6 ± 0.23°</td>
<td>15.5 ± 0.28°</td>
<td>2.8 ± 0.07°</td>
</tr>
<tr>
<td>5</td>
<td>8.6 ± 0.32°</td>
<td>12.8 ± 0.47°</td>
<td>2.8 ± 0.07°</td>
</tr>
<tr>
<td>6</td>
<td>8.0 ± 0.26°</td>
<td>11.8 ± 0.08°</td>
<td>2.6 ± 0.08°</td>
</tr>
</tbody>
</table>

° P < 0.01 versus others.

TABLE 4. The V_max of Ca^{2+} ATPase and Na^+K^+ATPase in pancreatic islet membranes isolated from normal rats and rats with different durations of CRF

<table>
<thead>
<tr>
<th>Ca^{2+}ATPase</th>
<th>Na^+K^+ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmol of ATP/mg of protein/h</td>
<td>μmol of ATP/mg of protein/h</td>
</tr>
<tr>
<td>Normal CRF (wk)</td>
<td>13.8 ± 0.53 (N = 10)</td>
</tr>
<tr>
<td>1</td>
<td>18.5 ± 0.96° (N = 10)</td>
</tr>
<tr>
<td>2</td>
<td>16.1 ± 0.59° (N = 9)</td>
</tr>
<tr>
<td>3</td>
<td>10.8 ± 1.02° (N = 13)</td>
</tr>
<tr>
<td>4</td>
<td>11.3 ± 0.38° (N = 10)</td>
</tr>
<tr>
<td>5</td>
<td>10.2 ± 0.49° (N = 12)</td>
</tr>
<tr>
<td>6</td>
<td>9.7 ± 0.37° (N = 10)</td>
</tr>
</tbody>
</table>

° P < 0.01 versus normal.

° P < 0.05 versus normal.

° P < 0.01 versus normal and first and second weeks of CRF.

° P < 0.01 versus normal first, second, and third week of CRF.

The activation of this exchanger may then cause an accumulation of sodium inside the islet, an event that would stimulate the activity of Na^+K^+ATPase (28). Indeed, our data show that the V_max of Na^+K^+ATPase began to rise during the first week of CRF and reached a peak during the third week of CRF.

Despite an increased activity of the calcium pump(s) during the first 2 wk of CRF, calcium extrusion out of the islets thereafter appears to lag behind the increased entry of calcium mediated by PTH as the blood levels of the hormone continue to rise and the V_max of the Ca^{2+}ATPase began to decline. Consequently, the [Ca^{2+}]_i starts to rise during the third week of CRF and continues to increase thereafter.

This increased calcium burden in the islets would inhibit mitochondrial oxidation and, hence, ATP production (17–19). Indeed, both basal and glucose-stimulated ATP content and ATP/ADP ratio began to fall during the third week of CRF and continued to decline thereafter. It should be mentioned that other investigators (18) have shown that an acute rise in [Ca^{2+}]_i stimulates mitochondrial oxidation and ATP generation. These observations appear contrary to our suggestion that the rise in [Ca^{2+}]_i caused a decrease in ATP content. However, these studies deal with the effects of an acute rise in [Ca^{2+}]_i, whereas our studies reflect the effects of a chronic rise in the basal level of [Ca^{2+}]_i.

Because ATP is required for normal function of Ca^{2+}ATPase and Na^+K^+ATPase (13,14,20), the decrease in ATP content during the third week of CRF could result in impaired activity of these enzymes. Our data are consistent with this notion in that the V_max of Ca^{2+}ATPase was significantly reduced during the third week of CRF, that of Na^+K^+ATPase was reduced during the fourth week of CRF, and the V_max of both enzymes remained low thereafter as ATP content of islets continued to fall. It could be argued that the affinity of these enzymes for ATP is in the micromolar range, and therefore, the decrease in the millimolar range of ATP observed in our studies is not adequate to cause an impairment in the V_max of these enzymes. However, Niki et al. (20) also found...
that the activity of Na⁺-K⁺ATPase of the HIT-T15 β cells was progressively inhibited as ATP was lowered from 3.0 to 1.5 mM. Our data and those of Niki et al. (20) support the suggestion that the ATP pool is compartmentalized within the cell with the concentration of ATP in the submembrane pool being considerably lower than that in other cytosolic pools of ATP. The changes in the activity of both Ca²⁺-ATPase and Na⁺-K⁺ATPase could be due to changes in the number of enzyme units per cell, changes in the activity of each enzyme, or both. Our data do not differentiate between these possibilities.

These sequences of events in the metabolism of the islets of CRF rats underlie the decrease in their glucose-induced insulin secretion. The latter began to fall during the third week of CRF as the basal level of [Ca²⁺]ᵢ began to rise and the ATP content started to fall. Glucose-induced insulin secretion displayed further inhibition, thereafter, as the magnitude of the changes in [Ca²⁺]ᵢ and ATP content of the islets became more pronounced.

Available data indicate that ATP plays an important role in the process of insulin secretion. ATP facilitates the closure of ATP-dependent potassium channels (31,32), which is followed by cell depolarization (32,33) and subsequent activation of voltage-sensitive calcium channels (31,32). This latter event permits calcium entry into the islets, causing a rise in [Ca²⁺]ᵢ (32), which triggers cellular processes leading to insulin secretion. Corkey et al. (34) suggested that the ATP/ADP ratio is the factor that modulates the closure of the ATP-dependent potassium channels and, hence, plays an important role in insulin secretion. Therefore, a lower ATP content and/or ATP/ADP ratio during the exposure of the islets to glucose would cause an inadequate closure of the ATP-dependent potassium channels and, hence, a smaller rise in [Ca²⁺]ᵢ, which would cause only a small Ca²⁺ signal, leading to impaired insulin secretion. Indeed, our data show that the glucose-induced rise in [Ca²⁺]ᵢ is significantly reduced during the fifth and sixth weeks of CRF when insulin secretion was markedly reduced.

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REFERENCES

8. Berson JA, Yallow RS: Parathyroid hormone in plasma in adenomatous hyperparathyroidism,


