Impaired potassium-induced insulin secretion in chronic renal failure

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Impaired potassium-induced insulin secretion in chronic renal failure. Extrarenal disposal of potassium load is impaired in chronic renal failure (CRF). This has been attributed to excess PTH since extrarenal disposition of potassium is normal in CRF-PTX animals. Insulin augments potassium entry into cells and hyperkalemia stimulates insulin secretion. Since glucose-induced insulin secretion is impaired in CRF and normal in CRF-PTX, it is possible that K⁺-induced insulin secretion is also impaired in CRF due to excess PTH. Such a defect would contribute to the abnormality in extrarenal disposal of potassium in CRF. We examined K⁺-induced insulin secretion, cytosolic calcium ([Ca²⁺]i) and the changes in [Ca²⁺]i in response to 20 mM KCl of islets from normal, CRF, and CRF-PTX rats; and normal and CRF animals treated with verapamil (normal-V and CRF-V). K⁺-induced insulin secretion by islets isolated from CRF rats was significantly (P < 0.01) lower than that from normal, CRF-PTX, CRF-V and normal-V rats. Basal level of [Ca²⁺]i in islets of CRF rats was significantly (P < 0.01) higher than in islets of the other four groups of animals. The calcium signal (Δ[Ca²⁺]i) and the Δ[Ca²⁺]i/basal [Ca²⁺]i ratio in response to 20 mM KCl observed in islets from CRF rats were significantly lower than in the other four groups of animals. The data indicate that: 1) K⁺-induced insulin secretion in islets of CRF is impaired, most likely, due to elevated basal level of [Ca²⁺]i and reduced calcium signal and/or smaller Δ[Ca²⁺]i/basal [Ca²⁺]i ratio in response to KCl; 2) the defect in K⁺-induced insulin secretion may contribute to the impaired extrarenal potassium disposal in CRF; and 3) the abnormalities in pancreatic islets may be mediated by the chronic excess of PTH in CRF.

Several studies have demonstrated that extrarenal disposal of a potassium load is impaired in both acute [1–3] and chronic renal failure (CRF) [4, 5]. Both aldosterone and epinephrine regulate extrarenal disposal of potassium [6], and a defect in these hormones in CRF could contribute to the abnormality in the reduced extrarenal disposal of this ion. However, aldosterone production is not reduced [7] and blood levels of catecholamines are elevated [8] in CRF.

Both acute [9] and chronic renal failure [10–12] are associated with secondary hyperparathyroidism and elevated blood levels of parathyroid hormone (PTH). This hormone enhances entry of calcium into cells [13–17] and chronic excess of PTH with or without CRF is associated with a rise in the basal levels of cytosolic calcium ([Ca²⁺]i) in many cells [12, 18–20]. Since the permeability of cellular membrane to potassium is affected by cytosolic calcium ([Ca²⁺]i) [21–23], it is possible that excess PTH by inducing a rise in [Ca²⁺]i is a major factor underlying the impairment in extrarenal disposal of potassium in CRF. Indeed the studies of Sugarman and Kahn [2, 3] and ours [5] support this proposition in that prevention of secondary hyperparathyroidism in CRF by parathyroidectomy (PTX) or blockade of the excessive PTH-induced entry of calcium into cells by a calcium channel blocker corrected the abnormality in extrarenal potassium handling [5].

However, excess PTH in CRF may interfere with extrarenal disposal of a potassium load through another pathway. Potassium stimulates insulin release from the pancreas [24] and many studies have demonstrated that insulin is an important regulator of extrarenal disposal of potassium [6]. Since secondary hyperparathyroidism of CRF impairs glucose-induced insulin secretion [12, 18, 25], it is possible that it also interferes with potassium-induced insulin secretion which would contribute to the overall process underlying the derangement of extrarenal potassium handling in CRF. The present study was designed to examine the effect of CRF with and without excess PTH on potassium-induced insulin secretion by pancreatic islets.

Methods

Male Sprague-Dawley rats weighing 170–250 g were studied. They were fed normal rat chow diet (ICN Nutritional Biochemical, Cleveland, Ohio, USA) throughout the study and were allowed to drink ad libitum. Experiments were performed in five groups of animals: a) normal rats, b) rats with CRF of 42 days duration, c) CRF-PTX rats maintained normocalcemic d) CRF rats treated with verapamil (0.1 µg/g body wt) given subcutaneously twice a day from day one of CRF (CRF-V), e) normal rats treated with verapamil for 42 days (normal-V). CRF was produced by 5/6 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 electrocautery, 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 5/6 nephrectomy. Two days before the sacrifice of the animals, they were housed in metabolic cages to obtain two consecutive, 24-hour urine collections for the measurement of
Potassium-induced insulin secretion was examined by dynamic perfusion studies of pancreatic islets. The details of this technique have been reported previously from our laboratory [25]. Twenty size-matched islets were used in each study. First, the islets were perfused with Krebs-Ringer bicarbonate buffer (pH = 7.4) containing 2.8 mM glucose and 3.5 mM KCl for 30 minutes. Thereafter, the potassium concentration was increased to 20 mM and the perfusion was continued for additional 30 minutes. Effluent was collected for six minutes prior to the change in potassium concentration and for 30 minutes thereafter for a total of 21 collections. Insulin secretion began to increase two minutes after the change of potassium concentration. Therefore, the average value of insulin release during the six minutes prior to the change in potassium concentration and the two minutes immediately thereafter were used as baseline level. The changes in insulin secretion from baseline with time were examined by calculating area under the curve for each study. This calculation allowed us to estimate insulin release during the initial phase (8 min including 3rd through 10th minutes) which is known to increase in response to potassium.

It should be mentioned that the concentrations of potassium (20 mEq/liter) used in the perfusate of the pancreatic islets were high and did not correspond with potential changes of potassium in vivo. However, the choice of 20 mEq/liter of potassium was made since lower concentrations of potassium elicited small changes in insulin secretion. Gomez and Curry [24] found that increasing potassium concentration to 8, 12 mEq/liter produced very little change in insulin secretion. We also found in preliminary studies of static incubations [25] that increasing potassium concentration to 8, 12, and 20 mEq/liter produced an increase in insulin secretion of 5.5 ± 2.6, 8.1 ± 3.1, and 15.0 ± 3.7, pg/islet/min, respectively.

Cytosolic calcium of the pancreatic islets was measured with fura 2/AM (Sigma Chemical Co., St. Louis, Missouri, USA) as described by Komatsu et al [27]. This technique utilizes entire islets and not dispersed islets cells as we have reported previously [12]. One hundred and fifty islets, isolated from the pancreas of one rat, were loaded with fura 2/AM by incubation in 2 μM of fura 2/AM for 30 minutes in an incubation medium containing the following in mM: 128 NaCl, 3.5 KCl, 1 MgCl2, 1.2 KH2PO4, 1.0 CaCl2, 1.25 NaHCO3, 20 HEPES, 2.8 glucose and 5 mg/ml BSA (pH 7.4). To remove the unincorporated probe, the islets were centrifuged for three to five seconds in an Eppendorf microfuge and washed twice and finally suspended in 2 ml of the incubation media. Measurement of fluorescence was done with Perkin-Elmer fluorescence spectrophotometer model LS-5B (Perkin-Elmer, Norwalk, Connecticut, USA) at excitation wavelength of 340 nm and emission wavelength of 510 nm. Komatsu et al found that the fluorescence signal of the preparation of the islet remains stable for at least a period of 25 minutes at excitation wavelength of 340 nm and emission wavelength of 510 nm. We also examined this issue in our studies and found the fluorescence signal of our preparation to be stable for at least 30 minutes, indicating that no leak of dye occurred over this period of time. After recording a steady state, 10 μl of 3.4 mM KCl stock was added to the 2 ml islet suspension to raise the KCl concentration to 20 mM and the fluorescence was recorded for 20 minutes. Maximal fluorescence (Fmax) and minimal fluorescence (Fmin) were estimated as previously reported [19]. The islets were lysed by 0.07% triton X-100 to obtain the Fmax; subsequently 5 mM EGTA (pH 13.2) was added to obtain the Fmin. Islets were washed before each experiment and the above-mentioned calibration for fura 2 signal was performed after each experiment. To eliminate the effect of autofluorescence due to cuvette, medium and unloaded 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 utilizing the Grynkiewicz equation: [Ca2+]i=Kd (F-Fmin)/(Fmax-F), [27, 28]. The dissociation constant (Kd) for Ca2+-Fura 2 was assumed to be 225 nm. Both basal [Ca2+]i and the effect of 20 mM KCl on [Ca2+]i were evaluated.

The measurements of calcium and magnesium concentration in plasma were made by Perkin-Elmer atomic absorption spectrophotometer, Model 503 and those of creatinine and phosphorus by Technicon autoanalyzer (Technicon Instrument Inc., Tarrytown, New York, USA). Plasma potassium was measured with IL flame photometer (Instrumentation Laboratory, Watertown, Massachusetts, USA). Insulin was determined by charcoal-coated radioimmunoassay using rat insulin as standard [29]. Serum level of PTH was determined by INS-PTH immunoassay kit (Nichols Institute Diagnostics, San Juan, Capistrano, California, USA). This assay recognizes the aminoterminal fragment of PTH. The lowest detectable level is 3 pg/ml, the interassay variation is 7.3% and intraassay variation is 4%.

Statistical analysis was done with the Clinfo computer system. The data are presented as mean ± SE. Changes in parameters with multiple measurements with time were evaluated by calculating the area under the curve for each experiment utilizing the trapezoidal rule. The significance of the differences in the data of the various groups was made by one-way analysis of variance and Duncan multiple range test.

Results

The biochemical data in the five groups of animals studied are given in Table 1. The 5/6 nephrectomy was associated with significant (P < 0.01) elevation in the concentration of creatinine in plasma with the values being three to four times higher than normal. There were significant (P < 0.01) reductions in creatinine clearance in CRF, CRF-PTX and CRF-V rats as compared to values in normal and normal-V rats. The values of creatinine clearance in CRF, CRF-PTX and CRF-V rats were not different. The plasma concentrations of calcium and phosphorus in the five groups were not different. Plasma potassium in CRF rats was significantly (P < 0.01) higher than all other groups and it was normal in CRF-PTX and CRF-V rats; these observations were similar to those previously reported from our laboratory [5]. 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 groups.
Table 1. Biochemical variables in the five groups of rats at the time of sacrifice

<table>
<thead>
<tr>
<th>Weight g</th>
<th>Creatinine mg/dl</th>
<th>Calcium mg/dl</th>
<th>Phosphorus mg/dl</th>
<th>Plasma potassium mEq/liter</th>
<th>Plasma PTH pg/ml</th>
<th>Creatinine clearance μl/min/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>212 ± 6</td>
<td>0.41 ± 0.02</td>
<td>9.4 ± 0.2</td>
<td>4.20 ± 0.10</td>
<td>18 ± 1.6</td>
<td>828 ± 69</td>
</tr>
<tr>
<td>CRF</td>
<td>178 ± 6</td>
<td>1.50 ± 0.22</td>
<td>9.5 ± 0.5</td>
<td>5.20 ± 0.10</td>
<td>51 ± 6.8</td>
<td>233 ± 18</td>
</tr>
<tr>
<td>CRF-PTX</td>
<td>210 ± 13</td>
<td>1.32 ± 0.20</td>
<td>9.6 ± 0.4</td>
<td>4.15 ± 0.15</td>
<td>10 ± 0.6</td>
<td>220 ± 27</td>
</tr>
<tr>
<td>Normal-V</td>
<td>208 ± 13</td>
<td>0.43 ± 0.03</td>
<td>9.3 ± 0.3</td>
<td>4.25 ± 0.10</td>
<td>21 ± 0.6</td>
<td>690 ± 46</td>
</tr>
<tr>
<td>CRF-V</td>
<td>188 ± 7</td>
<td>1.36 ± 0.20</td>
<td>8.7 ± 0.5</td>
<td>4.10 ± 0.18</td>
<td>72 ± 17.7</td>
<td>292 ± 54</td>
</tr>
</tbody>
</table>

*a P < 0.01 vs. normal and normal-VER

b P < 0.01 vs. all other groups

Potassium-induced insulin secretion during dynamic perfusion studies of islets from normal, CRF and CRF-PTX rats is shown in Figure 1. Early insulin release (8 min) calculated from area under the curve in CRF rats (57 ± 10.2 pg/islet · 8 min) was significantly ($P < 0.01$) lower than in normal (200 ± 36.4 pg/islet · 8 min) or CRF-PTX (189 ± 31.2 pg/islet · 8 min) rats. The values between normal and CRF-PTX rats were not different. Treatment of CRF rats with verapamil corrected the abnormality in insulin secretion by pancreatic islets isolated from these rats (Fig. 2). The area under the curve in CRF-V rats (185 ± 22.5 pg/islet · 8 min) was significantly ($P < 0.01$) higher than that in CRF rats and not different from those in normal and CRF-PTX rats. Verapamil treatment of normal rats did not affect insulin secretion by islets isolated from these rats with area under the curve, being 200 ± 40.6 pg/islet · 8 min.

Figure 3 depicts the basal levels of cytosolic calcium in the various groups studied, and Table 2 provides the change in [Ca$^{2+}$]i and ratio between the Δ[Ca$^{2+}$]i/basal [Ca$^{2+}$]i in response to 20 mM KCl in the five group of animals. The values of basal [Ca$^{2+}$]i in islets of normal rats (89 ± 3.4 nM) were not different from those (72 ± 10.1 nM) reported by Komatsu et al [27]. The basal levels of [Ca$^{2+}$]i in islets of CRF rats were significantly ($P < 0.01$) higher than those in the islets of the normal and normal-VER rats.
other four groups of animals. The Δ [Ca²⁺]i and the Δ [Ca²⁺]i/basal [Ca²⁺]i ratio in islets from CRF rats in response to 20 mm KCl were significantly (P < 0.01) lower than in the other four groups of animals. Although the Δ [Ca²⁺]i/basal [Ca²⁺]i ratio in the islets from normal-V and CRF-V rats were not different from those in islets of normal rats, they were lower than values in islets from CRF-PTX rats.

Discussion

Our data, as those of others [24], show that potassium stimulates the initial phase of insulin secretion from islets isolated from normal rats, and further demonstrate that the potassium-induced insulin secretion is significantly (P < 0.01) reduced in islets isolated from CRF rats. This derangement is prevented by prior PTX of the CRF rats or by their treatment with the calcium channel blocker, verapamil.

Potassium-induced insulin secretion is mediated by a rise in [Ca²⁺]i [30—32]. Potassium depolarizes the islet membrane and allows calcium to enter the islet, resulting in an acute rise in [Ca²⁺]i, which in turn triggers cellular events that leads to insulin secretion [32]. It has been suggested that an appropriate response of cells to agonists requires adequate calcium signal (Δ [Ca²⁺]i) and/or Δ [Ca²⁺]i/basal [Ca²⁺]i ratio [33]. Therefore, a smaller Δ [Ca²⁺]i and/or Δ [Ca²⁺]i/basal [Ca²⁺]i ratio could be associated with impaired insulin secretion. Our data show that both the Δ [Ca²⁺]i and the Δ [Ca²⁺]i/basal [Ca²⁺]i ratio in islets of CRF rats and their insulin secretion in response to KCl are significantly lower than in the other four groups. Our observations are, therefore, consistent with the proposition that CRF with excess PTH causes a sustained elevation in basal [Ca²⁺]i, and the latter is associated with a reduced calcium signal and a smaller Δ [Ca²⁺]i/basal [Ca²⁺]i ratio in response to KCl, and hence reduced insulin secretion. PTX of the CRF rats or their treatment with verapamil, by preventing the chronic persistent rise in basal [Ca²⁺]i, allows an adequate calcium signal and high Δ [Ca²⁺]i/basal [Ca²⁺]i ratio and hence normal insulin secretion. A corollary to our studies in pancreatic islets is found in observation in human polymorphonuclear leucocytes (PMNL) from dialysis patients and high blood levels of PTH [19]. The PMNL in these patients had elevated basal levels of [Ca²⁺]i and displayed smaller calcium signal and Δ [Ca²⁺]i/basal [Ca²⁺]i ratio and reduced physiological response to the ligation of their Fc γ RIII receptors to 3G8 monoclonal antibody.

The mechanisms responsible for the elevation in basal levels of [Ca²⁺]i in islets of CRF rats are complex and have been previously defined by us [12]. They involved augmented calcium entry, inhibition of mitochondrial oxygen consumption, reduced ATP content, and impaired calcium extrusion secondary to reduced activity of Ca²⁺ ATPase [12].

The demonstration that potassium-induced insulin secretion is reduced in CRF indicate that this defect in insulin secretion contributes, at least partly, to the impaired extrarenal disposal of a potassium load in CRF. These data provide another pathway through which excess PTH in CRF may participate in the reduced extrarenal disposition of potassium.

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References