Phosphate Depletion Reduces Potassium-Induced Insulin Secretion (43313)

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Abstract. **Potassium-induced insulin secretion is impaired in rats with chronic renal failure and a sustained rise in cytosolic calcium ($[Ca^{2+}]_i$). It has been found that the calcium signal ($\Delta[Ca^{2+}]_i$) and the $\Delta[Ca^{2+}]_i$/basal $[Ca^{2+}]_i$ in these animals in response to potassium are smaller than those in normal rats and that these defects may underlie, at least in part, the reduced potassium-induced insulin secretion, since the latter depends on an appropriate rise in $[Ca^{2+}]_i$. Since phosphate depletion (PD) is another model associated with a rise in the basal level of $[Ca^{2+}]_i$ of pancreatic islets, it provides another metabolic setting for investigating the interaction between high $[Ca^{2+}]_i$ of islets and their response to potassium. We examined the potassium-induced insulin secretion, the potassium-induced calcium signal, and the $\Delta[Ca^{2+}]_i$/basal $[Ca^{2+}]_i$ in islets of PD rats with and without elevated $[Ca^{2+}]_i$. The levels of the basal $[Ca^{2+}]_i$ in the islets of PD rats were significantly ($P < 0.01$) higher than those in pair-weighed (PW) animals and those in PD and PW rats treated with verapamil, which has been shown to prevent the rise in $[Ca^{2+}]_i$ in islets of PD rats. Both initial and total insulin secretion, the calcium signal, and the $\Delta[Ca^{2+}]_i$/basal $[Ca^{2+}]_i$ in the islets of PD rats were significantly ($P < 0.01$) smaller than those in the other three groups of animals. There were no significant differences in basal levels of $[Ca^{2+}]_i$ and in calcium signal, $\Delta[Ca^{2+}]_i$/basal $[Ca^{2+}]_i$, and insulin secretion among PW rats, verapamil-treated PD rats, and verapamil-treated PW rats. The results are consistent with the notion that elevated resting levels of $[Ca^{2+}]_i$ interfere with the magnitude of the calcium signal and the ratio of calcium signal to basal $[Ca^{2+}]_i$, and these derangements, at least in part, underlie the impaired potassium-induced insulin secretion in PD.** [P.S.E.B.M. 1991, Vol 198]

Several metabolic derangements in pancreatic islets, including reduced ATP content, elevated resting level of cytosolic calcium ($[Ca^{2+}]_i$), and impaired glucose metabolism, have been observed in chronic renal failure (1). These abnormalities were associated with impaired insulin secretion in response to glucose (1, 2) or potassium (3). Phosphate depletion (PD) is another experimental model in which the pancreatic islets display metabolic defects similar to those seen in chronic renal failure (4). Furthermore, glucose-induced insulin secretion is also impaired in PD (4). Studies with another insulin secretagogue, such as potassium, will help to further characterize the mechanisms of the defect in insulin secretion in PD.

Potassium-induced insulin secretion is mediated by a rise in $[Ca^{2+}]_i$ (5–7). It is possible that the sustained elevation in $[Ca^{2+}]_i$ of pancreatic islets in PD interferes with the magnitude of the calcium signal ($\Delta[Ca^{2+}]_i$) and/or the ratio between the calcium signal and basal $[Ca^{2+}]_i$, and hence results in reduced insulin secretion.

The present study examined insulin secretion and the magnitude of the calcium signal and the ratio between the calcium signal and basal $[Ca^{2+}]_i$ in pancreatic PD islets with and without elevated $[Ca^{2+}]_i$ after their exposure to potassium.

Methods

Animal Preparation. Male Sprague-Dawley rats weighing 180–225 g were used. The animals were divided into four groups. Two of the groups were fed a low-phosphate diet (with a phosphorus content of 0.03%; ICN Biochemicals, Cleveland, OH) and were designated PD rats; the other two groups received the control diet (phosphorus content, 0.99%; Wayne Re-

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search Animal Diets, Bartonville, IL) in a quantity adjusted to maintain their body weight equal to that of the PD animals; these two groups were designated pair-weighed rats (PW). The other components of the two diets were comparable (protein, 20% vs 24%; fat, 4.5% vs 4.5%). Calcium content was 0.6% in the PD diet versus 1.48% in the normal diet. The PD diet should contain lower calcium content. Otherwise, marked hypercalcemia may develop and endanger the life of the animal. The rats had free access to deionized water at all times. One group of the PD rats and one group of the PW rats were treated with verapamil (Isoptin Knoll AG, Ludiwigschafen, FRG) from Day 1 of the study. Verapamil was injected subcutaneously in a dose of 0.1 μg/g body wt twice daily; these animals were designated verapamil-treated PD rats (PD-V) and verapamil-treated PW rats (PW-V).

The animals were sacrificed by decapitation after 6 weeks of PD or verapamil treatment and the pancreases were removed and dissected free of adipose tissue and lymph nodes. Islets of Langerhans were isolated by the collagenase digestion method of Lacy and Kostianovsky (8) and picked free of exocrine tissue under a dissecting microscope.

Measurement of Insulin Secretion. The insulin secretions from the islets of PD, PW, PD-V, and PW-V animals were evaluated under dynamic conditions, as described previously (1, 2). In these studies, the first six collections (6 min) represented the basal level of insulin release during perifusion with 3.5 m*M* KCl. Thereafter, the KCl concentration in the perifusate was increased to 20 m*M* and an additional 30 samples were collected; insulin concentrations were determined in the various samples of the effluent. In all of the studies, the changes from baseline with time were examined by calculating the area under the curve for each study. The calculations of the areas under the curve allowed us to estimate insulin release during the initial phase (10 min, between minutes 3 and 12) and the total insulin release (27 min, between Minutes 3 and 30).

Measurement of Cytosolic Calcium. $[Ca^{2+}]_i$ of the pancreatic islets was measured with Fura 2/AM (Sigma Chemical Co., St. Louis, MO) as described by Komatsu *et al.* (9). This technique utilizes entire islets and not dispersed islet cells, as we have reported previously (1). One hundred and fifty islets, isolated from the pancreas of one rat, were loaded with Fura 2/AM by incubation in 2 μ*M* of Fura 2/AM for 30 min in an incubation medium containing the following: 128 m*M* NaCl, 3.5 m*M* KCl, 1 m*M* $MgCl_2$, 1.2 m*M* KH_2PO4, 1.0 m*M* $CaCl_2$, 1.25 m*M* $NaHCO_3$, 20 m*M* HEPES, 2.8 m*M* glucose, and 5 mg/ml of bovine serum albumin (pH 7.4). To remove the unincorporated probe, the islets were centrifuged for 3 to 5 sec in an Eppendorf microfuge, washed twice, and suspended in 2 ml of the incubation media. Measurement of 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. After reaching a steady state, 10 μl of 3.4 *M* KCl stock was added to the 2-ml islet suspension to raise the KCl concentration to 20 m*M*, and the fluorescence was recorded for 20 min. Maximal fluorescence and minimal fluorescence were estimated as reported previously (10). The islets were lysed by 0.07% Triton X-100 to obtain the F_{max}; subsequently, 5 m*M* EGTA (pH 13.2) was added to obtain the F_{min}. 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. The calculation of cytosolic calcium was made utilizing the Grynkiewicz equation:

$$[Ca^{2+}]_i = K_d\,(F - F_{min})/(F_{max} - F)$$

(9, 11). The dissociation constant for Ca^{2+}-Fura 2 was assumed to be 225 n*M*. Both the basal $[Ca^{2+}]_i$ and the effect of 20 m*M* KCl on $[Ca^{2+}]_i$ 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, NY). Insulin was determined by charcoal-coated radioimmunoassay using rat insulin as standard (12).

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 the Duncan multiple range test.

Results

Table I depicts the changes in body weight and the plasma concentrations of phosphorus, calcium, and magnesium in PD and PW rats with and without treatment with verapamil. All animals gained small amounts of weight during the study and there were no significant differences in their weights. The plasma concentration of phosphorus fell ($P < 0.01$) in both PD and PD-V rats and the values in the two groups of animals were not different at the time of sacrifice. There was also a modest fall in plasma phosphorus in PW and PW-V rats, but the values in PD and PD-V rats were significantly ($P < 0.01$) lower than those in PW and PW-V animals. The plasma concentration of calcium increased significantly ($P < 0.01$) to hypercalcemic levels

Table I. Body Weight and Plasma Values of Calcium, Phosphorus, Magnesium, and Creatinine in the Four Groups of Rats

	PD	PW	PD-V	PW-V
Body weight (g)				
Baseline	198 ± 1.4	194 ± 1.7	198 ± 1.2	192 ± 1.9
2 Weeks	225 ± 1.5	223 ± 0.9	230 ± 1.6	222 ± 2.1
4 Weeks	252 ± 4.9	247 ± 3.9	261 ± 1.9	255 ± 5.4
6 Weeks	266 ± 4.2	278 ± 6.4	269 ± 3.5	275 ± 10.6
Plasma phosphorus (mg/dl)				
Baseline	7.9 ± 0.06	8.1 ± 0.06	7.8 ± 0.13	7.9 ± 0.10
2 Weeks	6.6 ± 0.26	8.1 ± 0.12	5.8 ± 2.3	8.2 ± 0.10
4 Weeks	4.2 ± 0.06	7.3 ± 0.13	4.6 ± 0.18	7.4 ± 0.09
6 Weeks	4.1 ± 0.7	7.6 ± 0.12	4.4 ± 0.19	7.4 ± 0.06
Plasma calcium (mg/dl)				
Baseline	10.1 ± 0.06	10.1 ± 0.09	10.3 ± 0.09	10.0 ± 0.06
2 Weeks	11.6 ± 0.75	9.9 ± 0.08	12.6 ± 0.24	9.7 ± 0.13
4 Weeks	12.7 ± 0.20	9.9 ± 0.07	12.9 ± 0.10	10.8 ± 0.16
6 Weeks	12.1 ± 0.24	9.8 ± 0.10	11.9 ± 0.06	10.4 ± 0.12
Plasma magnesium (mg/dl)				
Baseline	2.2 ± 0.03	2.4 ± 0.10	2.1 ± 0.05	2.3 ± 0.04
2 Weeks	1.2 ± 0.05	1.9 ± 0.04	1.4 ± 0.05	2.0 ± 0.07
4 Weeks	1.4 ± 0.03	2.5 ± 0.03	1.8 ± 0.08	2.3 ± 0.17
6 Weeks	1.3 ± 0.06	2.2 ± 0.25	1.5 ± 0.06	2.3 ± 0.11
Plasma creatinine (mg/dl)				
At sacrifice	0.45 ± 0.02	0.44 ± 0.01	0.47 ± 0.03	0.42 ± 0.02

in both PD and PD-V rats. The plasma concentration of magnesium decreased significantly ($P < 0.01$) in PD and PD-V rats. At the time of sacrifice, the concentration of plasma creatinine was normal in all animals.

The potassium-induced insulin secretion during the dynamic studies is shown in Figure 1. Both early and total insulin secretions from the islets of PD rats were significantly impaired. The areas under the curve of early insulin release (3–12 min) in PD rats (70 ± 13.5 pg/islet.10 min) and of total insulin release (184 ± 45.2 pg/islet.27 min) were significantly ($P < 0.01$) lower than those in PW rats (220 ± 24.1 pg/islets.10 min and 553 ± 41.3 pg/min.27 min). Chronic treatment of PD rats with verapamil prevented the impairment in both early (263 ± 57.0 pg/islet.10 min) and total (765 ± 219.7 pg/islets.27 min) insulin secretions. Verapamil treatment of PW rats did not adversely affect initial (300 ± 47.6 pg/islets.10 min) or total (742 ± 144.9 pg/islets.27 min) insulin secretion from their isolated islets.

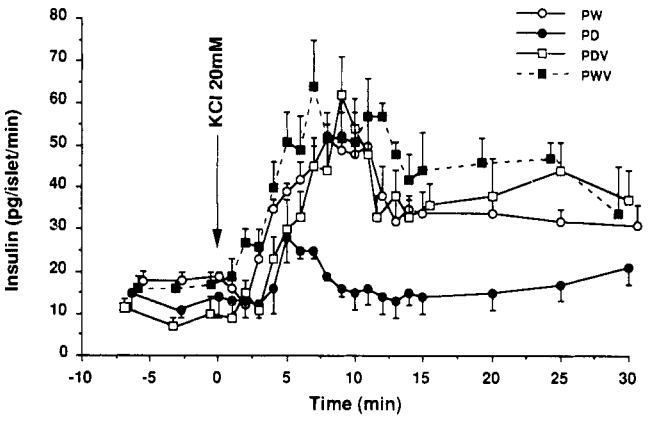

Figure 1. Dynamic studies of potassium-induced insulin release from perifused pancreatic islets of six PD, five PW, five PD-V, and five PW-V rats. The glucose concentration of the perifusate was 2.8 m*M* throughout the study and, at Time 0, the concentration of potassium was increased from 3.5 to 20 m*M*. Each datum point represents the mean value and the brackets depict 1 SE. The areas under the curve of both the initial and total insulin secretion by islets of PD rats are significantly ($P < 0.01$) smaller than those in normal or CRF-PTX rats.

Figure 2 provides the values of the resting levels of $[Ca^{2+}]_i$ of pancreatic islets and Table II shows the changes in $[Ca^{2+}]_i$ and the ratio between the $\Delta[Ca^{2+}]_i$ and basal $[Ca^{2+}]_i$ in response to 20 m*M* KCl in the four groups of animals. The values of basal levels of $[Ca^{2+}]_i$ in the islets of PW rats (84 ± 4.5 n*M*) are not different from those (72 ± 10.1 n*M*) reported in normal rats by Komatsu *et al.* (9). In islets isolated from the PD rats, the basal level of $[Ca^{2+}]_i$ (140 ± 8.4 n*M*) is significantly ($P < 0.01$) higher than that in PW (84 ± 4.5 n*M*), PD-V (88 ± 6.1 n*M*), and PW-V (85 ± 3.9 n*M*) rats. The $\Delta[Ca^{2+}]_i$ and the $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio in islets from PD rats in response to 20 m*M* KCl are significantly ($P < 0.01$) lower than those in the other three groups of animals.

Discussion

The results of the present study show that potassium-induced insulin secretion by pancreatic islets isolated from PD rats is impaired, and this derangement is prevented by treatment of PD rats with verapamil.

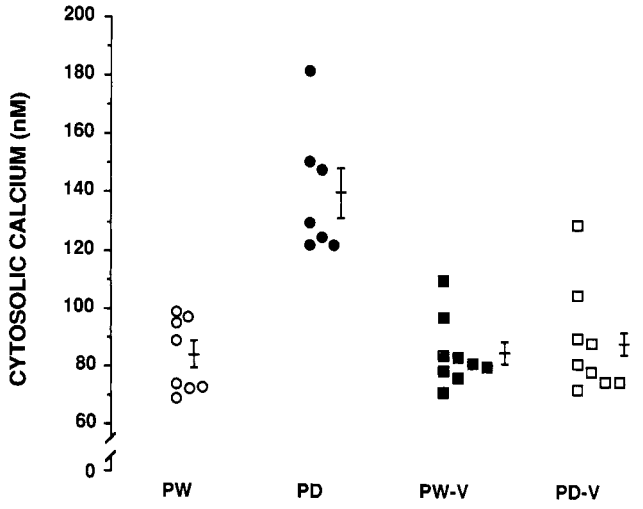

Figure 2. Basal levels of $[Ca^{2+}]_i$ in the four groups of animals studied. Each datum point represents one animal. Brackets denote mean ± 1 SE. The values in PD animals are significantly ($P < 0.01$) higher than those in the other three groups of rats.

Table II. Effect of 20 m*M* KCl on $[Ca^{2+}]_i$ of the Islets Isolated from the Various Groups of Rats

	Basal $[Ca^{2+}]_i$ (n*M*)	$\Delta[Ca^{2+}]_i$ KCl (n*M*)	$\frac{\Delta[Ca^{2+}]_i}{\text{Basal } [Ca^{2+}]_i}$ Ratio
PW ($n = 8$)	84 ± 4.5	84 ± 9.3	1.02 ± 0.07
PD ($n = 7$)	140 ± 8.4[a]	52 ± 7.0[a]	0.37 ± 0.05[a]
PD-V ($n = 9$)	88 ± 6.1	99 ± 13.3	1.14 ± 0.16
PW-V ($n = 9$)	85 ± 3.9	88 ± 14.3	1.02 ± 0.14

[a] $P < 0.01$ vs all other groups.

These observations are similar to those reported previously by us (4, 13) in regard to another insulin secretagogue, glucose, in that glucose-induced insulin secretion was also reduced in islets isolated from PD rats and treatment of these animals with verapamil almost completely corrected this defect.

PD is associated with reduced ATP content, a sustained rise in resting levels of $[Ca^{2+}]_i$, and impaired glucose metabolism of islets. The mechanisms of these derangements have been evaluated in detail by us (4, 13). Each or any combination of these metabolic abnormalities of the pancreatic islets could underlie the impairment in their insulin secretion in response to the two secretagogues, i.e., potassium and glucose.

Insulin secretion in response to potassium is mediated by a rise in $[Ca^{2+}]_i$ (5–7). This ion depolarizes the membrane of the pancreatic islets and allows calcium to enter the islets, resulting in an acute rise in $[Ca^{2+}]_i$ that in turn triggers cellular events that lead to insulin secretion. It has been suggested that an adequate calcium signal ($\Delta[Ca^{2+}]_i$) or a $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio in response to an agonist is required to initiate an appropriate physiological response (14). Therefore, a smaller $\Delta[Ca^{2+}]_i$ or $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio generated by KCl in PD islets could be associated with impaired insulin secretion. Our data show that both the $\Delta[Ca^{2+}]_i$ and the $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio in PD islets and their insulin secretion in response to 20 m*M* KCl are significantly lower than in the other groups. These observations are, therefore, consistent with the proposition that PD causes a sustained rise in basal $[Ca^{2+}]_i$ and that the latter is associated with reduced insulin secretion. Further support for this notion is provided by our finding that treatment of PD rats with verapamil, by preventing the chronic and persistent rise in basal $[Ca^{2+}]_i$, allowed an adequate calcium signal and an appropriate $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio and normal insulin secretion in response to KCl.

Similar observations were made in pancreatic islets isolated from rats with chronic renal failure (15). In these islets, the basal $[Ca^{2+}]_i$ was elevated and both the calcium signal and the $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio and insulin secretion in response to KCl were significantly lower than those in islets from normal rats. Furthermore, correction of the elevation in $[Ca^{2+}]_i$ in the islets of chronic renal failure rats by parathyroidectomy or treatment with verapamil was associated with normal basal $[Ca^{2+}]_i$ and calcium signal, $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio, and insulin secretion.

Another corollary to our results in pancreatic islets of PD or chronic renal failure rats is found in observations in human polymorphonuclear leukocytes from dialysis patients and high blood levels of parathyroid hormone. The polymorphonuclear leukocytes in these patients had elevated basal levels of $[Ca^{2+}]_i$ and displayed a smaller calcium signal and $\Delta[Ca^{2+}]_i$ to basal $[Ca^{2+}]_i$ ratio and reduced physiological response to the ligation of their Fc-γ-RIII receptors to monoclonal antibody (16).

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