The Effect of Glucose and Calcium on Ca^{2+}-Adenosine Triphosphatase in Pancreatic Islets Isolated From a Normal and a Non–Insulin-Dependent Diabetes Mellitus Rat Model

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Regulation of calcium balance is important in the secretory function of pancreatic islets. Ca^{2+}-adenosine triphosphatase (ATPase) is altered in tissues of non–insulin-dependent diabetes mellitus (NIDDM) rats, and they have an impaired response to glucose, "glucose blindness." We propose that the glucose blindness of the diabetic islet is the result of defective cellular calcium metabolism. Since Ca^{2+}-ATPase activity is important in the regulation of calcium balance, we investigated the effect of glucose and/or calcium on Ca^{2+}-ATPase activity in pancreatic islets in vitro and compared it with the effect in freshly isolated islets from controls and from rats with NIDDM induced by streptozotocin neonatally. Islets were isolated using collagenase and were stored fresh or cultured up to 2 days in RPMI 1640 in the presence of different concentrations of glucose and calcium. Membrane Ca^{2+}-ATPase activity, insulin secretion, and insulin content were determined. Ca^{2+}-ATPase activity was 1.30 ± 0.20 μmol/L Pi/μg membrane protein in normal noncultured islets and 1.02 ± 0.15 in islets cultured in 5.6 mmol/L glucose. Ca^{2+}-ATPase activity progressively decreased to 0.56 ± 0.10 and 0.34 ± 0.14 μmol/L Pi/μg membrane protein when glucose was increased in the culture media to 16.6 and 27.7 mmol/L, respectively. Decreasing glucose to 2.8 mmol/L did not alter Ca^{2+}-ATPase activity. Increasing or decreasing the Ca^{2+} content of the media did not significantly change Ca^{2+}-ATPase activity. Islets isolated from NIDDM rats had lower basal Ca^{2+}-ATPase activity and insulin content compared with normal controls. Incubation of islets from diabetic rats in high glucose further decreased the Ca^{2+}-ATPase content, but incubation in low glucose did not reverse it. Insulin secretion was responsive to glucose and calcium in normal islets, but was suppressed in islets from diabetic animals. From these studies, we conclude that high glucose, but not calcium, decreases Ca^{2+}-ATPase activity in islets from normal rats. Islets from NIDDM rats with glucose blindness have decreased Ca^{2+}-ATPase activity, likely due to the glucose status. We suggest that this decreased Ca^{2+}-ATPase activity may contribute to the pancreatic islets' glucose blindness.

OUR GROUP and others have proposed the concept that abnormal cellular Ca^{2+} ($[Ca^{2+}]_i$) metabolism causes insulin resistance and impairs insulin secretion and may be a basic common pathology of the non–insulin-dependent diabetes mellitus (NIDDM) syndrome.[1-5]

In favor of this concept are the accumulating data from animal models of diabetes and from investigations in patients with diabetes revealing that abnormal $[Ca^{2+}]_i$ is a common defect in both insulin-dependent diabetes (type I) and NIDDM (type II).[1,6-8] Altered $[Ca^{2+}]_i$ metabolism and/or regulation has been observed in most cell types of patients and animals with diabetes. Occult increases in plasma total calcium levels have been described in NIDDM patients, while levels of plasma ionized calcium are decreased.[9]

A striking feature of the impaired $[Ca^{2+}]_i$ metabolism in diabetes is the wide spectrum of abnormalities observed. However, this heterogeneity may be explained by the complex interaction between the different mechanisms involved in $[Ca^{2+}]_i$ homeostasis. A defect in one mechanism may change the function of other $[Ca^{2+}]_i$-regulating mechanisms. Thus, identification of the initial defect in $[Ca^{2+}]_i$ homeostasis in diabetes is of major significance.

Since the defect in $[Ca^{2+}]_i$ homeostasis in diabetes appears to be a general defect, we suggested that abnormalities in $[Ca^{2+}]_i$ homeostasis also occur in β cells of the pancreatic islets, and since glucose-stimulated insulin secretion from pancreatic islets depends on increases in $[Ca^{2+}]_i$ concentration, such defects may be the cause of the "glucose blindness" that is the characteristic defect in the insulin secretory response observed in the pancreas of NIDDM patients.[10-12] In favor of this concept are the recent findings by Tsuji et al,[13] who observed that β cells from islets obtained from NIDDM rats that show glucose blindness do not respond with the normal increase in $[Ca^{2+}]_i$ in response to a glucose challenge. Similar findings were reported by Roe et al[14] in islets obtained from the db/db mouse. However, the mechanisms for these defects remain unknown. Previous studies from our laboratory demonstrated that islets incubated in high-glucose media developed glucose insensitivity.[15,16] This glucose blindness was augmented by increased media calcium and was partially reversible using calmodulin/Ca^{2+}-adenosine triphosphatase (ATPase) inhibitor.[15] This suggests a role for Ca^{2+} balance, specifically Ca^{2+}-ATPase dysfunction, in the pathogenesis of glucose blindness in NIDDM.[17]

We propose that the Ca^{2+}-ATPase enzymes in both the endoplasmic reticulum and the plasma membrane are altered in NIDDM. While this defect may be genetically determined in NIDDM, the function of these enzymes may be further deteriorated in conditions of chronic hyperglycemia. In this study, we evaluated the function of plasma membrane and endoplasmic reticulum Ca^{2+}-ATPase in normal islets and islets from the neonatally induced NIDDM rat exposed to varying glucose and calcium environments in culture.

MATERIALS AND METHODS

Neonatally induced NIDDM rats were prepared by injecting streptozotocin 90 mg/kg dissolved in citrate buffer (pH 4.5) into 2-day-old male Wistar rats. Control male rats were injected with the citrate buffer alone. The animals were returned to their mothers and maintained. After

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5 to 6 weeks of age, the rats were fasted, blood samples were taken, and the rats were administered a glucose load (1.0 g/kg). A second blood sample was taken after 1.0 hour, and animals with glucose greater than 300 mg/dL after 1.0 hour were characterized as NIDDM in our model. Normal and NIDDM rats were further maintained and used in the studies at 8 weeks.

Pancreatic Islet Isolation

The isolation of pancreatic islets were performed using the method of Lacy et al.[18] Briefly, this consists of anesthetizing the animal, followed by infusion of Hanks solution retrogradely into the ductal system via the bile duct to disrupt the acinar tissue. The pancreas is then removed, chopped into fine pieces, and digested using collagenase at 37°C in a shaker. The pancreatic digest is washed and placed on a Ficoll discontinuous gradient. The gradient containing pancreatic digest is centrifuged, and the islet containing the Ficoll layer is collected.[19]

The islets were harvested and cultured in RPMI 1640 containing glucose 2.8, 5.6, 16.6, and 27.7 mmol/L or calcium 0.42, 1.42, or 3.42 mmol/L for 24 and 48 hours. After this time, the islets were harvested, the media were collected for insulin determination, and the Ca^{2+}-ATPase activity of plasma membrane and endoplasmic reticulum was measured using methods described previously.[20] In other studies, islets were prepared from NIDDM rats and Ca^{2+}-ATPase activity was determined in freshly isolated islets compared with freshly isolated controls. Evaluation of glucose-induced changes was made by repeating studies in islets grown for 24 and 48 hours in high (27.7 mmol/L) glucose and then measuring the Ca^{2+}-ATPase activity. Insulin secreted in the media was measured using an insulin kit (AutoPak; ICN, Costa Mesa, CA). The data were analyzed using ANOVA or paired *t* test.

RESULTS

When normal and NIDDM rats were given a glucose load, plasma glucose increased from 109 ± 2.9 mg/dL to a mean of 150 ± 0.9 mg/dL in normals and from 188 ± 8.8 mg/dL to 377 ± 27 mg/dL in NIDDM rats (Fig 1A). Insulin increased in response to glucose in normals, but the already elevated insulin levels in diabetics did not increase in response to glucose (Fig 1B).

Ca^{2+}-ATPase activity in normal islets was linearly related to plasma membrane protein, and the activity could be effectively modulated by calcium in the media (Fig 2). When these islets from normal rats were cultured for 24 hours in varying glucose concentrations, there was a progressive decrease in islet Ca^{2+}-ATPase activity at the higher (16.6 and 27.7 mmol/L) concentrations (Fig 3A). Similar observations were made when the islets were cultured under these same conditions for 48 hours (Fig 3B). The amount of insulin secreted in the culture media by the islets increased with increasing glucose in the media (Table 1).

When we evaluated the effect of media calcium on Ca^{2+}-ATPase activity, we noted that increasing or decreasing the media calcium content did not significantly alter Ca^{2+}-ATPase activity (Fig 4).

When Ca^{2+}-ATPase activity was determined in freshly isolated islets from normal and NIDDM rats, we observed that Ca^{2+}-ATPase activity was decreased in islets from NIDDM rats compared with normals (Fig 5). Subsequent incubation of these islets in a high-glucose or low-glucose environment for 24 and 48 hours resulted in a small but significant decrease in the already suppressed enzyme activity at 48 hours. However, incubation in low glucose did not return Ca^{2+}-ATPase activity to normal levels (Fig 6). Insulin secretion by islets from NIDDM rats was less than the secretion by normals (Table 1).

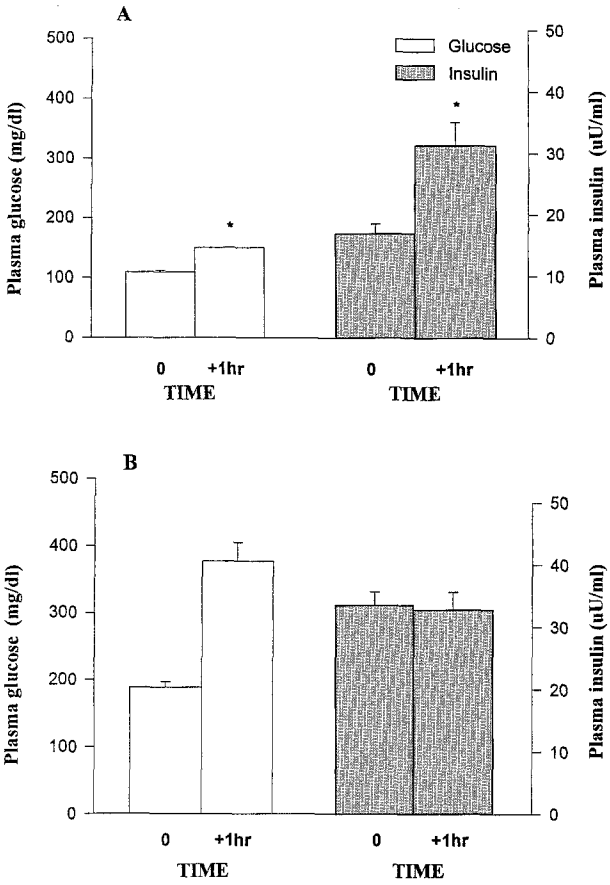

Fig 1. Plasma glucose (□) and insulin (▨) before and after a glucose load (1.0 g/kg) in fasted normal (A) and NIDDM (B) rats. *$P <$ .01 *v* fasting (n = 8 for both A and B).

DISCUSSION

These studies demonstrate that pancreatic islet Ca^{2+}-ATPase is sensitive to the prevailing glucose concentration in vitro, where increasing glucose decreases the enzyme activity. We also demonstrate that in our NIDDM rat model, diabetes is also

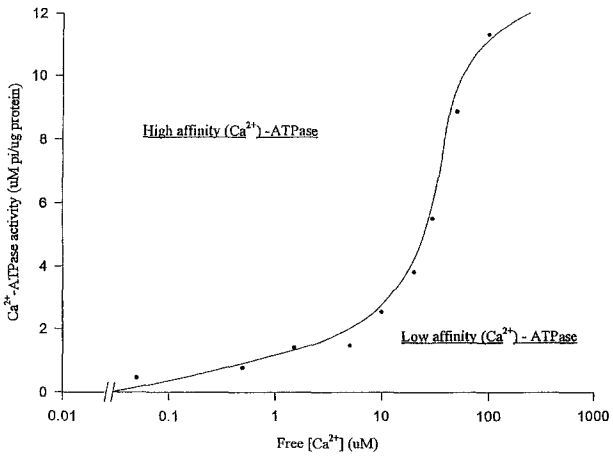

Fig 2. Ca^{2+}-ATPase activity in homogenates of freshly isolated islets. The curve represents Ca^{2+}-ATPase activity as a function of calcium concentration.

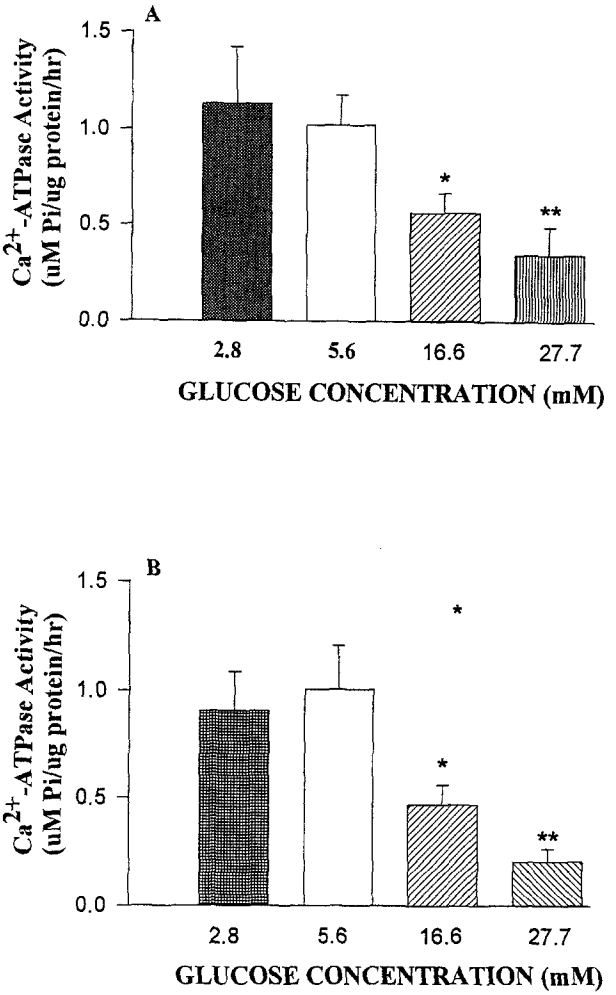

Fig 3. Ca^{2+}-ATPase activity in islets (4 μg protein/sample) following 24 (A) and 48 (B) hours of culture at different glucose concentrations (n = 6). *$P < .05$, **$P < .01$.

associated with a decreased Ca^{2+}-ATPase activity. These observations are consistent with previous studies demonstrating that obese diabetic animals are characterized by decreased Ca^{2+}-ATPase activity.[21] Studies on the acute effect of glucose on islet Ca^{2+}-ATPase also demonstrated that high glucose resulted in a transient decrease in the enzyme activity, which rapidly returned to baseline.[16] However, closer inspection showed a subsequent decline that would also support our observation. However, the observation that high glucose decreased Ca^{2+}-ATPase in islet tissue may be tissue-specific, in that previous

Table 1. Insulin Content and Insulin Secretion in Isolated Pancreatic Islets From Normal and NIDDM Rats

		Insulin Secretion (μU/islet/24 h)			
Group	Insulin Content (μU/islet)	Glucose 2.8 mmol/L	Glucose 5.6 mmol/L	Glucose 16.6 mmol/L	Glucose 27.7 mmol/L
Normal	875 ± 47 (6)	99.7 ± 16 (5)	124 ± 6 (5)	127 ± 24 (5)	150 ± 34 (5)
NIDDM	580 ± 32* (6)	46 ± 2.6* (5)	40 ± 11* (5)		59 ± 18* (5)

*$P < .01$.

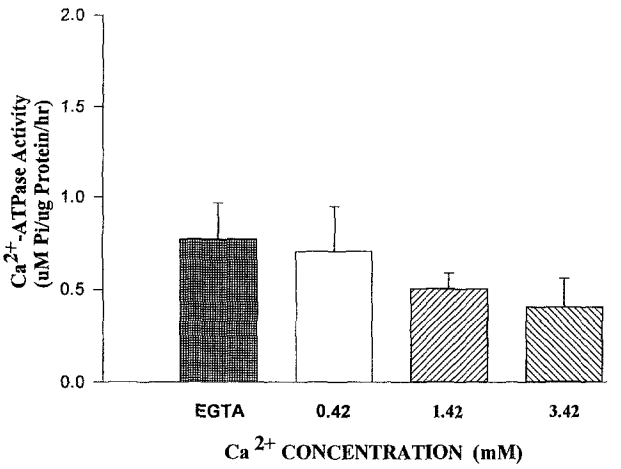

Fig 4. Ca^{2+}-ATPase activity in islets (4 μg protein/sample) following 24-hour culture in different calcium concentrations (media glucose was 5.5 mmol/L; n = 6).

investigations using this model have demonstrated increased Ca^{2+}-ATPase activity in other tissues such as kidney membranes and erythrocytes.[5,22-24]

Our observations support the hypothesis that cellular calcium overload is a major impairment in diabetes that may lead to glucose insensitivity. As we observed, hyperglycemia results in decreasing Ca^{2+}-ATPase activity, and this would result in a decreased ability to transport calcium out or decrease $[Ca^{2+}]_i$, resulting in a persistent elevated $[Ca^{2+}]_i$ and subsequent decreased response. The fact that significant suppression of Ca^{2+}-ATPase occurs in 24 to 48 hours is consistent with previous observations of declining glucose sensitivity in islets in a high-glucose environment.[15,16] The previously reported acute changes would also be consistent with the pattern of altered glucose sensitivity.[17]

When we evaluated Ca^{2+}-ATPase activity in the NIDDM rat model, we observed that the basal level of activity was already approximately half of what we observed in normal islets.

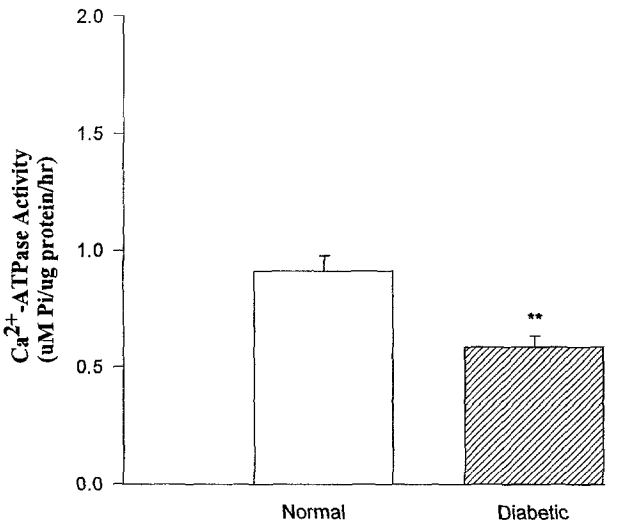

Fig 5. Ca^{2+}-ATPase activity in freshly isolated islets (4 μg protein/sample) from normal and NIDDM rats (n = 4). **$P < .05$.

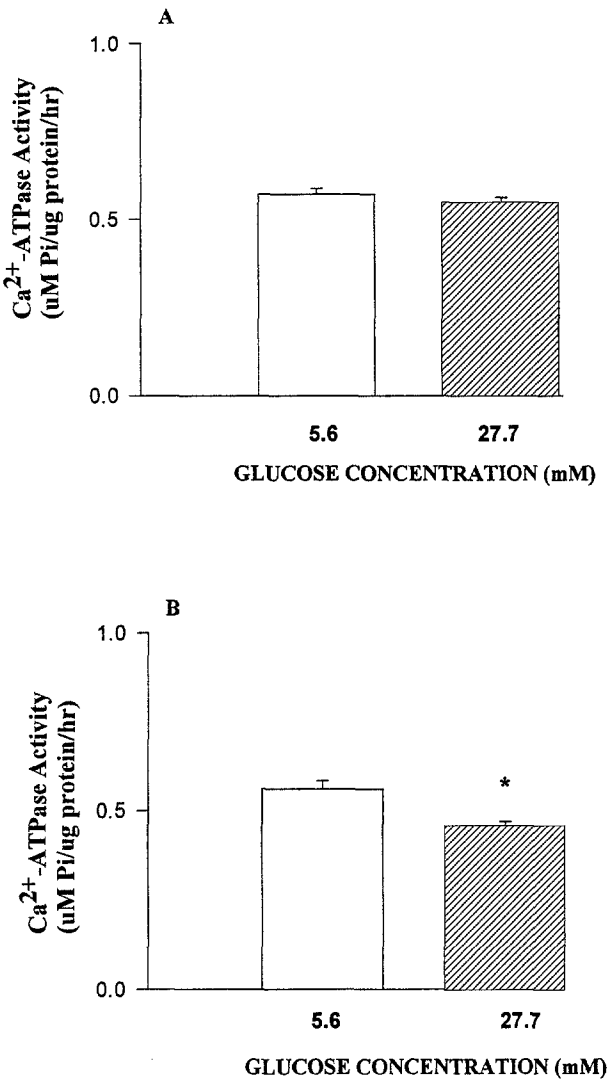

Fig 6. Ca^{2+}-ATPase activity in islets (4 μg protein/sample) from NIDDM rats cultured for 24 (A) and 48 (B) hours in different glucose concentrations (n = 4). *$P < .05$.

Subsequent exposure to a high-glucose environment was not effective after 24 hours, but it mildly decreased the activity after 48 hours' exposure, demonstrating that further suppression was possible. However, our observation that incubation in a normal glucose environment did not return Ca^{2+}-ATPase activity to normal levels suggests that the enzyme response is not rapidly reversible. We anticipated that the incubation time (48 hours) would allow recovery, and the 5.6-mmol/L glucose media maintained the highest activity in our normal islet studies.

The overall finding of decreased Ca^{2+}-ATPase is consistent with the lack of a significant insulin secretory response to the glucose challenge to determine the presence of diabetes in our animal models at 5 to 6 weeks of age. Our observation that basal insulin levels were already high when compared with normals but failed to increase in response to the glucose challenge is consistent with insulin resistance and glucose blindness. The decreased insulin secretion into the incubation media is also consistent with these observations and also supports previous observations.[16]

The finding that increases in media calcium did not significantly alter Ca^{2+}-ATPase activity was surprising, given that we previously observed a decreased islet responsiveness following prolonged calcium exposure.[15] Since it is well documented that glucose increases the $[Ca^{2+}]_i$ as a predecessor of insulin secretion,[25,26] it thus appears that the prevailing glucose environment, not the calcium concentration, is the significant regulator of enzyme activity. Other factors may be involved such as the prevailing insulin concentration or the insulin secreted into the media. Insulin itself has been suggested to increase Ca^{2+}-ATPase activity.[20] In our studies, the islet culture results in high insulin in the media, and this was true of the plasma in NIDDM animals. However, we do not believe this is a factor, because the absolute media insulin levels were high when the islets were incubated in a high- or moderate-glucose environment, as well as in studies where media calcium was varied. Another explanation may be that hyperglycemia, possibly via increasing diacylglycerol and protein kinase C, may act to decrease or increase Ca^{2+}-ATPase activity. However, we do not have data support for this possible mechanism.[27]

In this study, we have demonstrated that high glucose concentrations in culture media act to decrease Ca^{2+}-ATPase activity. And our NIDDM rat model using streptozotocin-treated newborn rats was insulin-resistant with glucose blindness, and this was also associated with decreased basal Ca^{2+}-ATPase activity. We conclude that decreased islet Ca^{2+}-ATPase in diabetic animals that results in increased cytoplasmic calcium may play a significant role in the decreased responsiveness to a glucose challenge.

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