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Low protein diet impairs glucose-induced insulin secretion from and ^{45}Ca uptake by pancreatic rat islets

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Glucose-induced insulin secretion from and ^{45}Ca uptake by isolated pancreatic islets, derived from rats fed with normal (NPD) or low protein diet (LPD), were studied. Insulin secretion from both types of islets in response to increasing concentrations of glucose followed an S-shaped pattern. However, basal secretion observed at substimulatory concentrations of glucose (0–5.6 mM), as well as maximal release, obtained at 16.7 mM or higher glucose concentrations were significantly reduced in islets from LPD. Furthermore, in LPD rat islets, the dose-response curve to glucose was clearly shifted to the right compared with NPD islets, with the half-maximal response occurring at 8.5 and 14.4 mM glucose for NPD and LPD islets, respectively. In islets from NPD rats, the ^{45}Ca content, after 5 or 90 min in the presence of 8.3 mM glucose, was higher than that observed for islets kept at 2.8 mM glucose and increased further at 16.7 mM glucose. After 5 min of incubation, the ^{45}Ca uptake by LPD islets in the presence of 8.3 mM glucose was slightly higher than basal values (2.8 mM glucose); however, no further increase in the ^{45}Ca uptake was noticed at 16.7 mM glucose. In LPD islets a significant increase in ^{45}Ca uptake over basal values was registered only at 16.7 mM glucose, after 90 min of incubation. These data indicate that the poor secretory response to glucose observed in islets from LPD rats may be related to a defect in the ability of glucose to increase Ca^{2+} uptake and/or to reduce Ca^{2+} efflux from β-cells. (J. Nutr. Biochem. 6:314–318, 1995.)

Keywords: protein deficiency; islets of Langerhans; insulin secretion; Ca^{2+} fluxes

Introduction

It is known that dietary deficiency causes altered carbohydrate metabolism in children, adults, and animals.[1–6] In general, protein calorie malnutrition is associated with a decreased glucose tolerance and reduced insulin secretion.[6–9] Since the pancreatic insulin content has been reported to be normal or even higher in animals maintained on a low protein diet,[6,10] the impaired insulin secretion in these animals cannot be ascribed to a reduction in the insulin content. Rather it could be a consequence of a reduction in the β-cell volume and/or a reduction in the number of glucoreceptors in these cells.[7,8,11] The present data indicate that the poor secretory response to glucose by islets isolated from rats maintained with a low protein diet may be related to defects in Ca^{2+} handling by these islets.

Methods and materials

Young (28-day-old) rats weighing approximately 90 g were distributed into two groups and fed for 8 weeks either with a normal (25%) or a low (6%) protein diet *(Table 1)*. The difference between the two isocaloric diets was the substitution of carbohydrate for protein in the low protein diet. The other components of both diets were kept unaltered.[12] At the end of the experimental period the nutritional status of the animals was evaluated by determina-

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Table 1 Composition of normal and low protein diets fed to rats during the experiment

Component	Composition (g/kg)	
	Normal protein	Low protein
Casein*	315	75
Starch	183	268
Glucose	141	248
Sucrose	150	203
DL-methionine	7	2
Corn oil	150	150
Salt mixture†	40	40
Vitamin mixture‡	10	10
Choline chlorhydrate	4	4

*Values corrected for protein content in casein.
†According to Rogers, Q.R. and Harper, H.E. (1965). *J. Nutr.* **87**, 267.
‡According to Miller, S.A. et al. (1962). *J. Nutr.* **77**, 397.

tion of body weight, total serum protein,[13] serum albumin,[14] serum glucose,[15] serum free fatty acids,[16] and liver glycogen content.[17]

To measure insulin secretion, groups of five islets each were first incubated at 37°C in a small volume (0.75 ml) of Krebs-bicarbonate solution containing 5.6 mM glucose for 30 min. The solution was then replaced by fresh buffer, and the islets were further incubated for 60 min under various experimental conditions. The insulin content of each sample was measured as previously described[18] using rat insulin as the standard. The glucose concentration producing a response that was 50% of the maximum (EC_{50}) was calculated as mean negative logarithms (pD_2).

To measure ^{45}Ca uptake by islets, we used a previously described method.[19] All results are expressed as mean ± SE together with the number of individual experiments (n). Statistical analysis included Student's t test and analysis of variance.

Results

Nutritional status of the animals

After 8 weeks, the LPD rats showed the following features: low body weight, hypoalbuminemia, and high liver glycogen content compared with the NPD rats. Despite low body weight registered in LPD animals, no difference in food intake was noticed during the experimental period between both groups of rats. No evidence of edema in the LPD animals was noticed. Furthermore, serum concentrations of protein, glucose, and free fatty acids did not differ between the two groups of rats *(Table 2)*.

Glucose-induced insulin secretion

Figure 1 shows that in both types of islets, glucose-induced insulin secretion followed an S-shaped pattern. Basal secretion, observed at 2.8 mM glucose, was 0.38 ± 0.05 and 1.04 ± 0.08 ng/islet/60 min ($P < 0.05$) in LPD and NPD islets, respectively. Maximal release, obtained at 16.7 mM glucose, was significantly higher than basal secretion in both types of islets (1.44 ± 0.08 and 5.84 ± 0.35 ng/islet/60 min in LPD and NPD islets, respectively); however, the rise in insulin secretion was greater in NPD than in LPD islets ($P < 0.01$). Furthermore, the dose-response curve to increasing concentration of glucose was shifted to the right in LPD compared with NPD islets with half maximal release values of 14.4 and 8.5 mM glucose, respectively ($P < 0.05$).

^{45}Ca uptake

At basal glucose (2.8 mM), the ^{45}Ca uptake was higher in NPD than LPD islets, averaging 0.93 ± 0.07 ($n = 23$) and 0.63 ± 0.03 ($n = 47$) pmol/islet/5 min, respectively ($P < 0.05$). In 8.3 mM glucose, ^{45}Ca incorporation increased to 1.38 ± 0.11 ($n = 24$) and 1.07 ± 0.15 ($n = 41$) pmol/islet/5 min in NPD and LPD islets, respectively ($P < 0.05$ related to each basal value). ^{45}Ca uptake by NPD islets increased further when the glucose concentration was raised to 16.7 mM, reaching 1.91 ± 0.13 ($n = 29$) pmol/islet/5 min ($P < 0.05$). However, in this experimental condition, no additional increment in the ^{45}Ca uptake was recorded in LPD islets *(Figure 2)*. After 90 min incubation *(Figure 3)*, ^{45}Ca content in LPD islets increased only at 16.7 mM glucose, to 3.8 ± 0.26 ($n = 46$) pmol/islet/90 min ($P < 0.05$ related to basal values). In NPD islets, ^{45}Ca uptake was 7.32 ± 0.32 ($n = 21$) and 8.18 ± 0.38 ($n = 26$) pmol/islet/90 min in the presence of 8.3 and 16.7 mM glucose, respectively ($P < 0.01$). These values were significantly higher than that observed at 2.8 mM glucose, which was 5.58 ± 0.41 ($n = 10$) pmol/islet/90 min ($P < 0.05$).

Discussion

Experimental model

Low body weight, hypoalbuminemia, and high liver glycogen content, observed in rats maintained with a low protein

Table 2 Body weight (g), food intake (g/g of body weight), serum total protein (g/L), basal serum glucose (mmol/L), serum free fatty acids (FFA = mmol/L), and liver glycogen content (mg%) of rats fed with low or normal protein diets during 8 weeks

Groups	Serum				Liver glycogen	Body weight	Food intake
	Glucose	FFA	Protein	Albumin			
NPD	5.7 ± 0.7	0.5 ± 0.1	68.2 ± 4.3	34.6 ± 0.7	0.7 ± 0.4	240.0 ± 30.0	7.4 ± 0.8
LPD	6.7 ± 1.5	0.5 ± 0.1	66.5 ± 2.8	32.7 ± 1.4*	1.3 ± 0.7*	120.0 ± 40.0*	7.4 ± 0.4

Results are mean ± SD for 10 rats in each group.
NPD = normal protein diet.
LPD = low protein diet.
*Significantly different from NPD

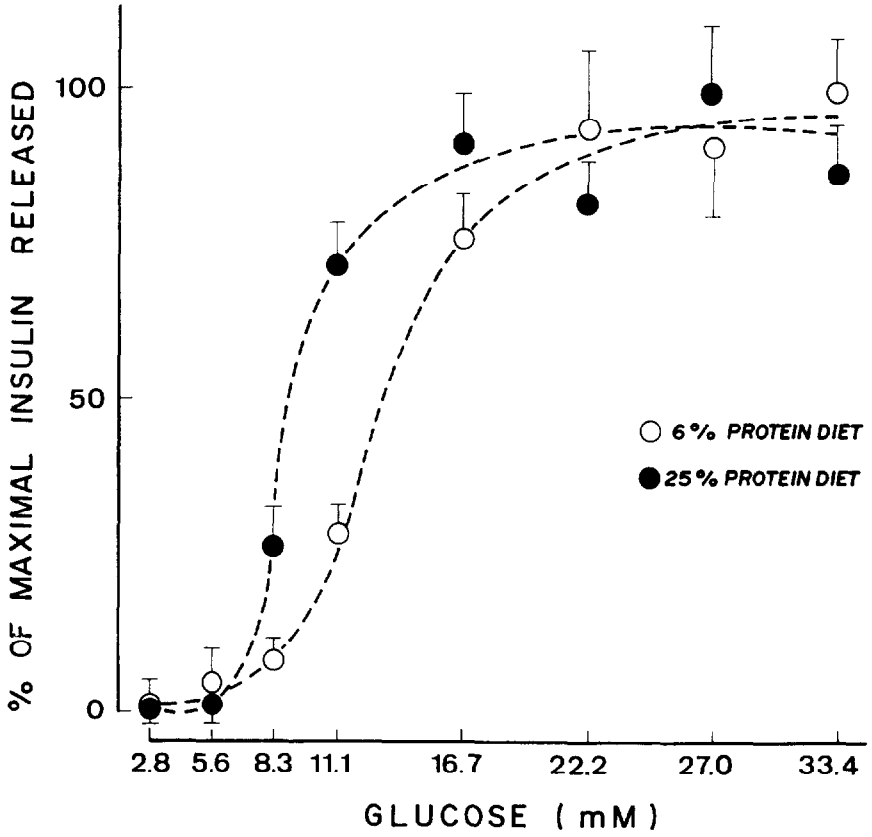

Figure 1 Steady-state dose-response relationship. Prior to the application of different concentrations of glucose, islets were preincubated for 30 min at 37°C. Next, the incubation medium was replaced with fresh Krebs solution containing increasing concentrations of glucose. Points represent cumulative (60 min) insulin secretion at the indicated concentration of glucose. Each point represents the mean value of the insulin released per islet ± S.E. together with the number of experiments, which was 12–23 and 14–29 for NPD (●---●) and LPD (○---○) islets, respectively.

diet, are features commonly found in infantile and experimental animals,[6,20,21] indicating the adequacy of the animal model used in this work. The absence of edema (a usual symptom in malnourished persons) in LPD animals was not surprising in view of the reported difficulties in producing edema in rats.[6,22]

Insulin release in response to increasing concentrations of glucose

It is known that protein calorie malnutrition results in carbohydrate intolerance. The elevated blood glucose level during a glucose tolerance test in malnourished persons may

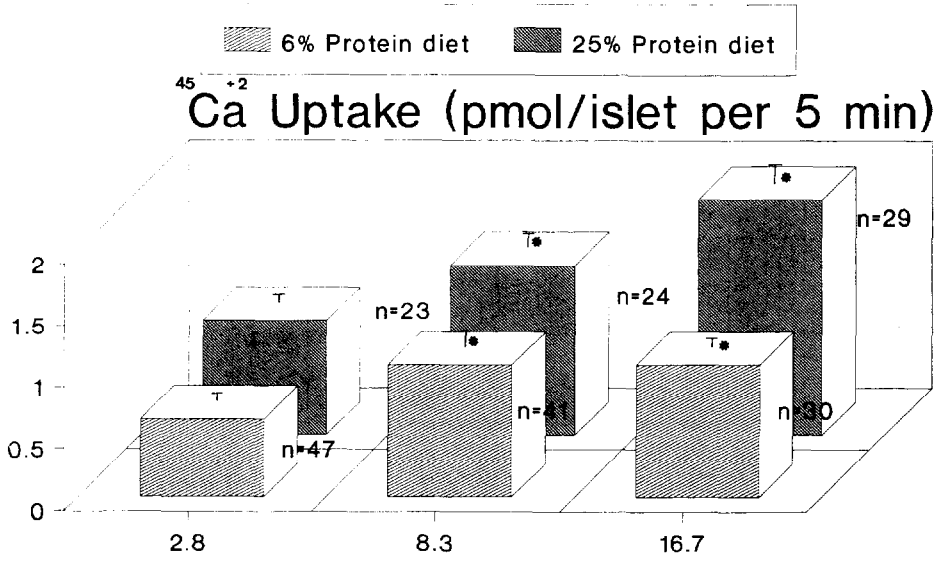

Figure 2 ^{45}Ca uptake by isolated islets after 5 min of incubation. Effects of increasing concentrations of glucose on ^{45}Ca uptake by NPD (filled bars) and LPD islets (dashed bars). Means ± S.E. together with the number of experiments are indicated. Asterisks indicate $P < 0.05$ compared with basal values measured in 2.8 mM glucose.

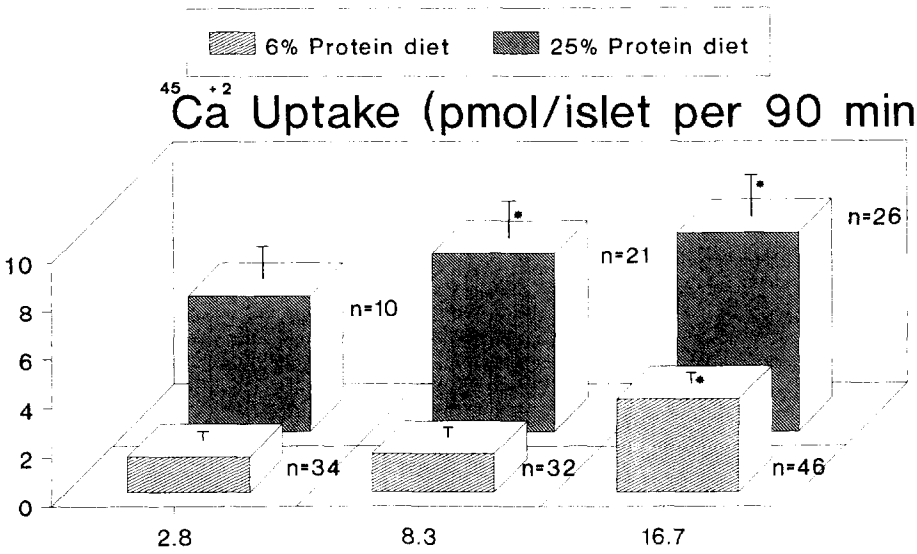

Figure 3 ^{45}Ca uptake by isolated islets after 90 min of incubation. Effects of increasing concentrations of glucose on ^{45}Ca uptake by NPD (filled bars) and LPD islets (dashed bars). Means ± S.E. together with the number of experiments are represented. Asterisks indicate $P <$ 0.05 compared with basal values measured in 2.8 mM glucose.

be due to decreased insulin secretion and/or to increased resistance to insulin.[1,4] In experimental models of protein energy malnutrition, glucose intolerance was clearly established only after a long period on a low-protein diet.[23] However, a severe reduction of insulin release in response to glucose is consistently noticed after a few weeks of exposure to a low-protein diet.[23,24] In these animals an increase in the sensitivity to insulin of peripheral tissues may explain why the animals do not develop glucose intolerance.[24]

Our data confirm that insulin secretion in response to glucose is reduced in malnourished rats. However, it is not clear if the decreased insulin secretion observed in LPD islets is due to problems related to glucose recognition,[11] or to defects in one or more steps in the cascade of events responsible for the extrusion of insulin-containing granules, or both. Although islet insulin content is similar or even higher in LPD than NPD islets,[6,10] one current explanation for the reduced response to glucose is that LPD islets are smaller than NPD islets.[8] Incidentally, it was reported that insulin secretion capacity parallels the size of β-cells.[8,25] However, differences in islet size and/or cell volume cannot explain the reduced sensitivity to intermediate concentrations of glucose found in the LPD islets *(Figure 1)*. In these islets, the dose-response curve to glucose was shifted to the right, indicating that one (or more) intrinsic defect in the mechanism of insulin secretion is involved.

^{45}Ca accumulation in response to glucose

Stimulation of insulin secretion by glucose depends on an increase in $[Ca^{2+}]_i$ in β-cells. Glucose affects $[Ca^{2+}]_i$ mainly by increasing its membrane permeability, but also by decreasing the Na^+/Ca^{2+} exchange and by mobilizing Ca^{2+} from internal stores.[26] Thus, we decided to investigate whether Ca^{2+} handling is altered in LPD islets. Our results indicate that the influx of ^{45}Ca, based on measurements in a short period of time (5 min), as well as ^{45}Ca retention after 90 min of incubation, were altered in LPD islets. In particular, at intermediate concentrations of glucose, where insulin secretion was clearly impaired, no differences in Ca^{2+} uptake related to basal values were noticed in LPD islets.

β-cell regulates $[Ca^{2+}]_i$ within a very narrow window.[27] Insulin secretion is only noticed when a threshold for $[Ca^{2+}]_i$ is attained. Because the ^{45}Ca uptake, at 2.8 mM glucose, is much higher in NPD than LPD islets, one could argue that a small increase in Ca^{2+} uptake, induced by 8.3 mM glucose over basal, will be more efficacious in reaching $[Ca^{2+}]_i$ threshold values for insulin secretion in NPD than in LPD islets. Higher concentrations of glucose (i.e., 16.7 mM) provoke additional secretion whereas $[Ca^{2+}]_i$ is only marginally increased. It was recently postulated that high glucose must exert actions independent of depolarizing β-cell membrane to account for this amplification process.[28] This effect seems to depend on the energy state of the cell[29] and could involve phosphoinositide hydrolysis and the consequent generation of IP_3 and activation of protein kinase C.[30] Since glucose-induced PI hydrolysis depends on Ca^{2+} influx in the β-cell,[31] this could explain why insulin secretion is impaired at both intermediate and high glucose concentrations in LPD islets.

In conclusion, our studies with isolated islets indicate that both Ca^{2+} influx and efflux are altered in LPD islets. Therefore, the poor secretory response to glucose by the LPD islets, at least in part, may be due to altered Ca^{2+} handling by these islets. However, we have to keep in mind that the abnormalities in Ca^{2+} handling may be a consequence of alterations in one or more steps that precedes the modifications in Ca^{2+} permeability during insulin secretion, such as glucose transport, glucose metabolism, and K^+ permeability. Alterations in nutrient metabolism in malnourished islets could affect the cellular redox state with reduction of the thiol content and glutation (reduced form)/

glutation (oxidized form) ratio. This in turn alters cellular Ca^{2+} homeostasis[32,33] and insulin secretion, as already observed.[33]

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References

1 Bowie, M.D. (1964). Intravenous glucose tolerance in Kwashiorkor and Marasmus. *S. Afr. Med. J.* **38,** 328–329

2 Baig, A.A. and Edozien, J.C. (1965). Carbohydrate metabolism in Kwashiorkor. *Lancet* **II,** 662–665

3 Rao, K.S.J. (1965). Kwashiorkor and Marasmus, blood sugar levels and responses to epinephrine. *Am. J. Dis. Child.* **110,** 519–521

4 James, W.P.T. and Coore, H.G. (1970). Persistent impairment of insulin secretion and glucose tolerance after malnutrition. *Am. J. Clin. Nutr.* **23,** 386–389

5 Smith, S.P., Edgar, P.J., Pozefsky, T., Cheetri, M.K., and Prout, T.E. (1975). Insulin secretion and glucose tolerance in adults with protein-calorie malnutrition. *Metab. Clin. Exp.* **24,** 1073–1084

6 Weinkove, C., Weinkove, E., Timme, A., and Pimstone, B.L. (1977). Pancreatic islets of malnourished rats. *Arch. Pathol. Lab. Med.* **1012,** 66–269

7 Young, J.K. and Dixit, P.K. (1980). Lack of diabetogenic effect of alloxan in protein-calorie malnourished rats. *J. Nutr.* **110,** 703–709

8 Dixit, P.K. and Serensen, R.L. (1987). Effect of protein-calorie malnutrition on insulin secretion. *Indian J. Med. Res.* **86,** 663–670

9 Mello, M.A.R. and Cury, L. (1989). Effects of protein-calorie malnutrition on endocrine pancreatic function in young pregnant rats. *Brazilian J. Med. Biol. Res.* **22,** 791–794

10 Mello, M.A.R., Carneiro, E.M., Boschero, A.C. and Cury, L. (1990). Endocrine pancreatic function in young pregnant malnourished rats. Studies in vivo. *XVIII International Congress of the International Academy of Pathology*, p. 30

11 Dixit, P.K. and Kaung, H.L.C. (1985). Rat pancreatic B cell in protein deficiency: a study involving morphometric analysis and alloxan effect. *J. Nutr.* **115,** 375–381

12 Mello, M.A.R., Cury, L., Valle, L.B.S., and Oliveira-Filho, R.M. (1987). Pregnancy in young rats: effects of malnutrition. *Nutr. Rep. Int.* **30,** 527–535

13 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

14 Doumas, B.T., Watson, W.B., and Briggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta* **31,** 87–96

15 Nogueira, D.M., Strufaldi, B., Hirata, M.H., Abdalla, D.S.P., and Hirata, R.D.C. (1990). Sangue—Parte I: Glicidios. In *Metodos de Bioquimica Clinica* (D.M. Nogueira, B. Strufaldi, D.S.P. Abdalla, and R.D.C. Hirata, eds.), p. 153–168

16 Falholt, K., Luod, B., and Falholt, W. (1983). An easy colorimetric micromethod for routine determination of free fatty acids in plasma. *Clin. Chim. Acta*, **46,** 105–111

17 Hassid, W.Z. and Abrahan, S. (1957). Chemical procedures for analysis of polisaccharides. *Meth. Enzymol.* **3,** 34–36

18 Scott, A.M., Atwater, I., and Rojas, E. (1981). A method for the simultaneous measurement of insulin release and B-cell membrane potential in single mouse islets of Langerhans. *Diabetologia* **21,** 407–475

19 Boschero, A.C., Crepaldi, S.C., Carneiro, E.M., Delattre, E. and Atwater, I. (1993). Prolactin induces maturation of glucose sensing mechanisms in cultured neonatal rat islets. *Endocrinology* **133,** 515–520

20 Heard, C.R.C., Franji, S.M., Wright, P.M., and McCartney, P.R. (1977). Biochemical characteristics of different forms of malnutrition: an experimental model using young rats. *Br. J. Nutr.* **37,** 1–21.

21 Chaves, N. (1986). Principais carências nutricionais. In *Nutrição Básica e Aplicada* (N. Chaves, ed.), p. 292–313, Guanabara Koogan, Rio de Janeiro

22 Widdowson, E.M. and McCance, R.A. (1957). Effect of a low protein diet on chemical composition of the bodies and tissues of young rats. *Br. J. Nutr.* **11,** 198–206

23 Crace, C.J., Swenne, I., and Milner, R.D.G. (1989). Long-term follow-up after early protein-calorie malnutrition in young rats: sex difference in glucose tolerance and serum insulin levels. *Metabolism* **38,** 933–938

24 Okitolonda, W., Richard, S.M., Pottier, A.M., and Henquin, J.C. (1988). Influence of low- and high-protein diets on glucose homeostasis in the rat. *Brit. J. Nutr.* **60,** 509–516

25 Rao, R.H. (1990). Chronic undernutrition may accentuate the β-cell dysfunction of type II diabetes. *Diabetes Res. Clin. Pract.* **8,** 125–130

26 Wollhein, C.B. and Sharp, G.W.G. (1981). Regulation of insulin release by calcium. *Physiol. Rev.* **61,** 914–973

27 Rojas, E., Carroll, P.B., Ricordi, C., Boschero, A.C., Stojilkovic, S.S., and Atwater, I. (1994). Control of cytosolic free calcium in cultured human pancreatic β-cells occurs by external calcium-dependent and independent mechanisms. *Endocrinology* **134,** 1771–1781

28 Gembal, M., Silon, P., and Henquin, J.C. (1992). Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse β cells. *J. Clin. Invest.* **89,** 1288–1295

29 Gembal, M., Detimary, P., Gilon, P., Gao, Z.-Y., and Henquin, J.C. (1993). Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K^+ channels in mouse β cells. *J. Clin. Invest.* **91,** 871–880

30 Kelly, G.G., Zawalish, K.C., and Zawalish, W.S. (1994). Calcium and a mitochondrial signal interact to stimulate phosphoinositide hydrolysis and insulin secretion in rat islets. *Endocrinology* **134,** 1648–1654

31 Biden, T.J., Petre-Riesch, B., Schlegel, W., and Wollheim, C.B. (1987). Ca^{2+}-mediated generation of inositol 1,4,5-triphosphate and inositol 1,3,4,5-tetrakisphosphate in pancreatic islets. *J. Biol. Chem.* **262,** 3567–3571

32 Dwivedi, R.S., Gruebele, A., and Novak, R.F. (1992). Effects of altered calcium homeostasis on the expression of glutathione S-transferase isozymes in primary cultured rat hepatocytes. *Biochem. Pharmacol.* **44,** 2099–2103

33 Malaisse, W.J., Dufrane, S.P., Mathias, P.C.F., Carpinelli, A.R., Malaisse-Lagae, F., Garcia-Morales, P., Valverde, I., and Sener, A. (1985). The coupling of metabolic to secretory events in pancreatic islets. The possible role of glutathione reductase. *Biochem. Biophys. Acta* **844,** 256–264