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Glucose inhibits the high-affinity (Ca^{2+} + Mg^{2+})-ATPase in the plasma membrane of a glucose-responsive insulinoma

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(Ca^{2+} + Mg^{2+})-ATPase enzyme activity of a purified plasma membrane preparation from a glucose responsive rat insulinoma, was characterized as Ca^{2+}-dependent dephosphorylation of [γ-^{32}P]ATP. A high-affinity enzyme with a K_m(ATP) ranging from 20 to 30 μM and a K_m(Ca^{2+}) of 1 μM was identified. Glucose inhibited this high-affinity enzyme in a dose-dependent manner, with no significant inhibition at a concentration between 0 and 5 mM, 50% inhibition at 13.3 mM and 94.5% inhibition at 30 mM. The inhibitory effect of glucose was immediate and rapidly reversible. The effect was stereospecific for the α-anomer. These findings support the concept that glucose acts directly at the β-cell plasma membrane and is involved in the maintenance of elevated intracellular free calcium concentrations associated with insulin release by directly or indirectly inhibiting energy-dependent calcium efflux. Glyceraldehyde (20 mM) increased enzyme activity 3-fold, while other metabolic fuels had no effect. This suggests that inhibition of the enzyme is not an obligatory requirement for insulin release. Calmodulin stimulated the enzyme activity in calmodulin-depleted but not in undepleted membranes. Trifluoperazine (30–100 μM) inhibited (Ca^{2+} + Mg^{2+})-ATPase in a dose-dependent manner (14–61% activity) and the activity was also inhibited by vanadate (0.1–1.0 mM) and NaCl (150 mM).

Introduction

Calcium plays an important role in the regulation of insulin release from the β-cell. A rise in cytosolic free calcium has been implicated as a trigger of insulin release. Glucose stimulates the influx and initially inhibits the efflux of calcium [1,2] and leads to an increase in cytosolic free calcium concentrations [3]. The control of efflux at the plasma membrane may, therefore, be an important regulator of insulin release. Efflux of Ca^{2+} may be controlled by ion exchange mechanisms such as Na^+/Ca^{2+} and Ca^{2+}/Ca^{2+} exchange. It might also be regulated by the 'calcium pump' which is a (Ca^{2+} + Mg^{2+})-ATPase, although it has been shown recently that total (Ca^{2+} + Mg^{2+})-ATPase activity does not necessarily concur with total Ca^{2+}-transport activity [4–8]. This enzyme has been characterized in islet cell homogenate, subcellular fractions and a plasma membrane enriched fraction [9–11]. Glucose, the major physiological stimulus of insulin release, inhibited the enzyme at stimulatory and non-stimulatory concentrations in one study [11], but had no effect in another study [10]. Since a prompt inhibitory effect of glucose on the plasma membrane (Ca^{2+} + Mg^2)-ATPase was seen in human erythrocytes [12], we investigated the effect of glucose and other secretagogues on (Ca^{2+} + Mg^{2+})-ATPase activity in a glucose-responsive rat insulinoma, taking advantage of this plentiful source of islet cell material which allows purification of plasma membranes.

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Materials and Methods

Materials

[γ-^{32}P]ATP was obtained from ICN Pharmaceuticals (Irvine, CA). Calmodulin was obtained from Behring Diagnostic (San Diego, CA) and TFP was purchased from Aldrich Chemical Company (Milwaukee, WI).

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; CDTA, cyclohexane-1,2-diamine-*N,N,N',N'*-tetraacetic acid; Pipes, 1,4-piperazinediethanesulfonic acid; SDS, sodium dodecylsulfate; TFP, trifluoperazine dehydrochloride.

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Hanks' solution and culture medium were obtained from Gibco (Grant Island, NY). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). The α-D-glucose used contained less than 5% of the β-anomer, while β-D-glucose contained less than 2% of the α-anomer. Values for purity were supplied by manufacturer and confirmed by Dr. H.K. Desai, Department of Chemistry, University of Georgia (Athens, GA). Anomers were prepared and kept on ice until added to the assay mixture. A calcium ion electrode with reference electrode was obtained from Orion Research (Cambridge, MA).

Animals

A radiation-induced rat insulinoma which has been shown by electron microscopic examination to contain mainly β-cells, was transplanted under the right kidney capsule of male New England Deaconess Hospital (NEDH) rats weighing 300–400 g as described in detail elsewhere [13]. The tumor was maintained by serial transplantations in this strain of rats.

Tumor digestion and β-cell purification

The digestion procedure for tumor tissue has been described [3]. Each tumor yielded approx. $(100–200) \cdot 10^6$ cells with a viability of $> 95\%$ when tested for Trypan blue exclusion. One to two tumors were used for each experiment. The β-cells were then separated from the other cells by mixing the cell pellet with 6 ml of 27% (w/w) Ficoll in Hanks' buffer (pH 7.5). Over this were layered successively 3 ml each of Hanks' buffer containing 23.3, 20.5 and 12.2% (w/w) Ficoll (pH 7.5). The discontinuous gradient was centrifuged at $700 \times g$ for 30 min. The tissue from one tumor was generally applied to 4 gradients. The β-cells were harvested from the 12.2/20.5% interface, consolidated into a large tube and washed twice (40 ml each) with Hanks' buffer to remove the Ficoll.

Purification of plasma membranes

Purified plasma membranes were prepared according to the method by Naber et al. [14] with minor modifications. The plasma membrane fraction (B_1) was frozen at $-70\,^\circ$C, and used within 4 weeks without loss of activity. Protein was measured according to the method described by Bradford [15]. The plasma membrane marker enzyme 5′-nucleotidase was approximately 11-fold purified in the plasma membrane fraction (26.1 ± 2.8 and 278.2 ± 18.7 nmol/mg per min in homogenate and plasma membrane fraction respectively, $n = 16$). The endoplasmic reticulum marker enzyme NADH–cytochrome-*c* reductase activity ranged from 21.7 to 93.6 nmol/mg per min in the homogenate fraction, while it ranged from undetectable to 29.0 nmol/mg per min in the B_1 fraction.

Ca^{2+} measurements

$CaCl_2$ stock solutions were standardized using a Ca^{2+} specific electrode (Orion Research, Cambridge, MA). The endogenous Ca^{2+} and Mg^{2+} content of the plasma membrane preparations was measured by atomic absorption spectrometry. Free Ca^{2+} concentrations in the assay mixtures were buffered with EGTA, and were calculated using a computer program written according to Pershadsingh and McDonald using a Ca-EGTA association constant of $10^{7.832}$ at pH 7.5 [16].

Measurement of ($Ca^{2+} + Mg^{2+}$)-ATPase activity

($Ca^{2+} + Mg^{2+}$)-ATPase activity was determined as the Ca^{2+}-dependent dephosphorylation of [γ-^{32}P]ATP [10]. Briefly, the assay medium consisted of 50 mM Tris-Pipes (pH 7.5 at 37 °C), 20 mM NaN_3, 30 μM ATP (Mg salt), 1 μCi of [γ-^{32}P]ATP/tube and 0–2 mM EGTA ± $CaCl_2$. $CaCl_2$ was varied to give the required free Ca^{2+} concentrations. The endogenous Ca^{2+} concentrations varied from 0.56 to 0.58 nmol/mg protein, while the endogenous Mg^{2+} concentration ranged from 1.9 to 4.2 μmol/mg protein. Although generally 0.25 μg protein was used per reaction, the reaction rate was linear from 0.1 to 1 μg of protein (see Results). The final assay volume was 100 μl and the reaction was started by addition of ATP and terminated after 10 min by addition of 50 μl of 3% SDS followed by a double charcoal sedimentation method [17]. A 0.5 ml aliquot was removed from the supernatant fraction and counted in a scintillation counter (Beckman Instruments, Inc., Palo Alto, CA). In experiments investigating the effect of different secretagogues or other substances the free Ca^{2+} concentration used was 5.47 μM and the plasma membrane fractions were pre-incubated with the secretagogue for 30 min at 37 °C unless stated otherwise. The Ca^{2+}-dependent hydrolysis of [γ-^{32}P]ATP into ADP and [^{32}P]P_i was measured by subtracting the hydrolysis of ATP obtained in the presence of EGTA from that obtained in the presence of Ca^{2+} and EGTA.

Statistical analysis

All data are expressed as the mean ± S.E. unless otherwise stated. The significance of difference between means was determined by Student's *t*-test. *N* refers to the number of experiments done in triplicate from separate plasma membrane preparations.

Results

Characterization of the plasma membrane ($Ca^{2+} + Mg^{2+}$)-ATPase activity

The ($Ca^{2+} + Mg^{2+}$)-ATPase activity increased in a linear fashion with increasing amounts of plasma membrane protein, as is shown in Fig. 1. The activity of the enzyme was also linear with time (Fig. 2). Background ATPase activity was $26.5 \pm 1.4\%$ ($n = 42$) of that ob-

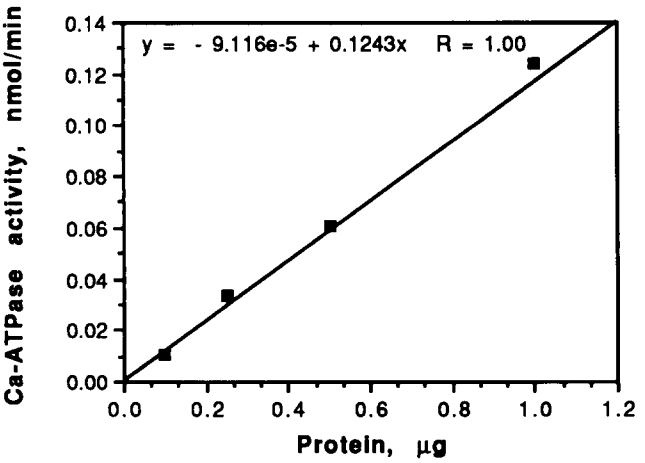

Fig. 1. Protein dependence of $(Ca^{2+} + Mg^{2+})$-ATPase activity. The enzyme activity was determined as the Ca^{2+}-dependent dephosphorylation of [γ-^{32}P]ATP at a free Ca^{2+} concentration of 5.47 μM at increasing protein concentrations.

tained with 5.47 μM free calcium. The analysis of Lineweaver-Burk plots were consistent with a low- and a high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase. In the presence of 5.47 μM free Ca^{2+}, the K_m for ATP ranged from 20.9 to 29.2 μM ($n = 3$) for the high-affinity (ATP) enzyme, while it was 427.7 $\pm$ 65.0 μM ($n = 3$) for the low-affinity (ATP) enzyme. At an ATP concentration of 30 μM, the K_m for Ca^{2+} ranged from 0.47 to 0.94 μM ($n = 2$). The high-affinity enzyme also was dependent upon the presence of magnesium. This was shown using the chelator CDTA as described elsewhere by Pershadsingh et al. [18]. A Ca-CDTA buffer system was used and compared to a Ca-EGTA buffer system. At equimolar concentrations CDTA reduced Ca^{2+}-stimulated enzyme activity completely (i.e., to baseline concentrations obtained with calcium chelator and no

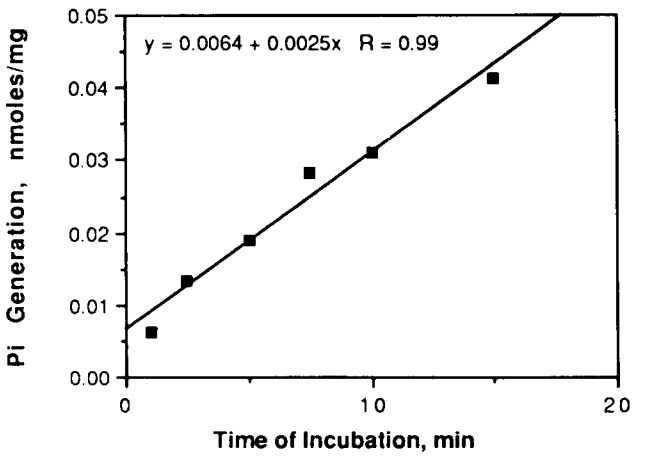

Fig. 2. Time dependence of $(Ca^{2+} + Mg^{2+})$-ATPase activity. The enzyme activity was determined as the Ca^{2+}-dependent dephosphorylation of [γ-^{32}P]ATP at a free Ca^{2+} concentration of 5.47 μM. The protein used per reaction was 0.25 μg. The reaction was started by addition of ATP and terminated at different time intervals by addition of 3% SDS as described in Materials and Methods.

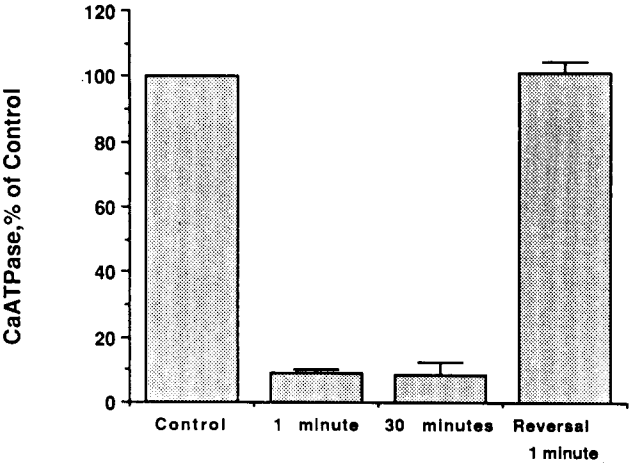

Fig. 3. The immediate inhibitory effect of 30 mM α-D-glucose on $(Ca^{2+} + Mg^{2+})$-ATPase is seen in less than 1 min and is not different at 30 min of exposure to high glucose. This inhibition is completely reversible in less than 1 min. The preincubation time of the membrane protein with glucose was varied from less than 1 min to 30 min in experiments investigating the rapidity of the glucose effect, while in experiment studying the reversibility of the effect, membrane protein was prepared in 30 mM glucose (A) or 3 mM glucose (B). Part of A was then diluted 10-fold, while part was used undiluted and aliquos of A and B were then assayed at various incubation times (less than 1 min to 30 min). The part of A that was diluted 10-fold and B showed no inhibition and were not different from the control which did not contain glucose.

calcium added) compared with the activity obtained in a Ca-EGTA buffer at the same free calcium concentrations.

Effect of glucose on plasma membrane $(Ca^{2+} + Mg^{2+})$-ATPase activity

When the plasma membranes were pre-incubated in the absence of 30 mM α-D-glucose, the enzyme specific activity was 71.9 $\pm$ 10.2 nmol/min per mg protein while 30 mM D-glucose decreased $(Ca^{2+} + Mg^{2+})$-ATPase activity to 4.89 $\pm$ 1.10 nmol/min per mg protein ($n = 14$, $P < 0.001$). The inhibitory effect of glucose on the enzyme activity was evident also in less than 1 min of pre-exposure to glucose (i.e., as fast as glucose could be added) and was not significantly different at 1 min of preincubation than at 30 min (91.0 $\pm$ 1.3% inhibition vs. 91.3 $\pm$ 3.6% inhibition, $n = 4$, Fig. 3). When the dose dependence of the inhibitory effect of glucose was investigated it was found that 2.5 and 5 mM glucose had no effect on $(Ca^{2+} + Mg^{2+})$-ATPase activity when compared to control, 15 mM glucose inhibited by 54.4 $\pm$ 6.7%, 20 mM inhibited by 89.3 $\pm$ 2.7%, and, at 30 mM glucose inhibited by 94.5 $\pm$ 1.7% ($n = 3$) (Fig. 4). Half-maximal inhibition of the enzyme was calculated to be at 13.3 mM glucose. The immediate inhibitory effect of glucose on the enzyme activity was rapidly reversible. In less than 1 minute after dilution of the glucose concentration (3 mM), the activity of $(Ca^{2+} + Mg^{2+})$-ATPase ranged from 97.4 to 104.8% of the con-

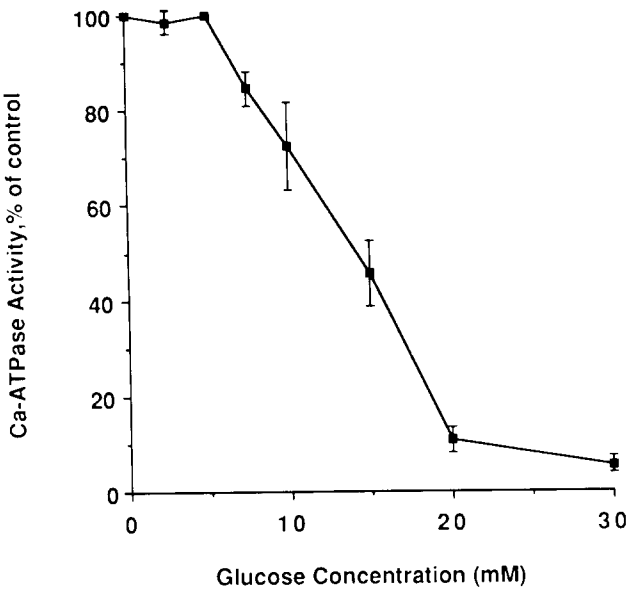

Fig. 4. Dose dependence of the inhibitory effect of glucose on $(Ca^{2+} + Mg^{2+})$-ATPase activity. The membrane protein was preincubated for 30 min with varying concentrations of α-D-glucose and $(Ca^{2+} + Mg^{2+})$-ATPase activity assayed as described in Materials and Methods.

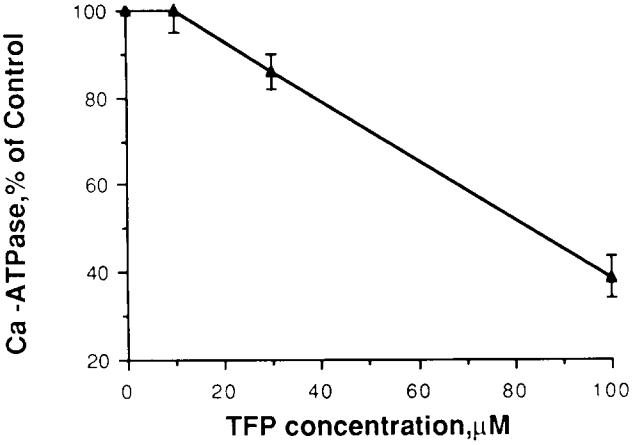

Fig. 5. Dose dependence of the inhibitory effect of TFP on $(Ca^{2+} + Mg^{2+})$-ATPase activity. The membrane protein was incubated with TFP for 30 min and $(Ca^{2+} + Mg^{2+})$-ATPase activity assayed as described in Materials and Methods.

trol ($n = 2$). The stereospecificity of the inhibitory effect of glucose on enzyme activity was then further investgated. α-D-Glucose and β-D-glucose (30 mM each) were either prepared and kept on ice until used in the assay or both anomers were allowed to equilibrate for 10 min at 37 °C. This method of sample handling was identical to that used when the composition of the glucose anomers was determined by polarimetry. After the 10 min equilibration period α-D-glucose constituted 46.2% and β-D-glucose 53.8% of total glucose concentration. When the anomers were kept on ice and added to the membranes, the inhibition of $(Ca^{2+} + Mg^{2+})$-ATPase activity seen with α-D-glucose ranged from 64.5 to 80.8% compared to the enzyme activity without addition of a secretagogue, while that seen with β-D-glucose ranged from 3.1 to 11.0% ($n = 2$). However, when both glucose anomers were allowed to equilibrate, inhibition seen with α-D-glucose was 38.4 and 33.9%, and was 36.1 and 42.2% with β-D-glucose.

Effect of other insulin secretagogues and modifiers of insulin secretion on plasma membrane $(Ca^{2+} + Mg^{2+})$-ATPase activity

20 mM DL-glyceraldehyde had a stimulatory effect on the activity of the $(Ca^{2+} + Mg^{2+})$-ATPase (176.8 ± 36.8% stimulation vs. control, $n = 5$; $P < 0.05$).

No effect on $(Ca^{2+} + Mg^{2+})$-ATPase activity was seen with 30 mM α-ketoisocaproate and with glucose 6-phosphate at 10 and 30 mM. No effect was also seen with 20 mM mannose, galactose, fructose, or mannoheptulose. The muscarinic agent carbachol (2 mM) did not affect the enzyme activity, nor did calmodulin at concentrations between 1 and 50 μg/ml in the presence or absence of EGTA at various calcium concentrations. However, in calmodulin-depleted membranes [19] $(Ca^{2+} + Mg^{2+})$-ATPase activity was stimulated 2-fold by addition of 25 μg/ml calmodulin (180.2 ± 6.8% of control, $n = 3$). 30 mM mannitol or 33% sucrose also did not lead to a change in enzyme activity, nor did ouabain (1 mM).

In addition, the effect of TFP and vanadate on $(Ca^{2+} + Mg^{2+})$-ATPase activity was studied as they will lead to inhibition of the plasma membrane $(Ca^{2+} + Mg^{2+})$-ATPase in other systems and might be used in the differentiation of enzyme origin i.e. plasma membrane or endoplasmic reticulum. In the present study, a decrease in the activity of $(Ca^{2+} + Mg^{2+})$-ATPase ac-

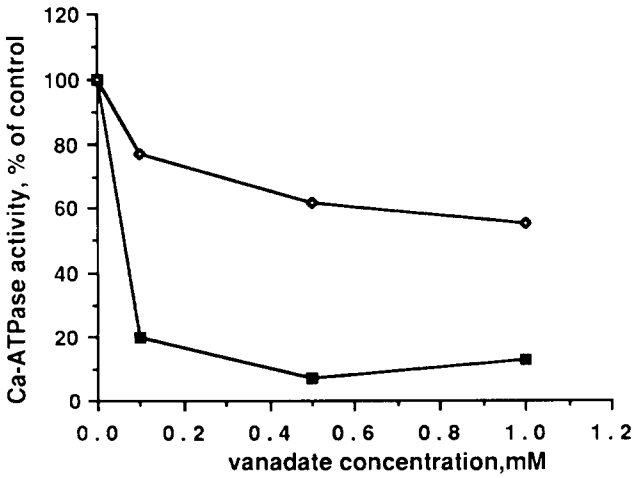

Fig. 6. Inhibitory effect of vanadate on $(Ca^{2+} + Mg^{2+})$-ATPase activity in the presence (■———■) and absence (◇———◇) of 2 mM $MgCl_2$. The membrane protein was incubated with vanadate in the absence and presence of 2 mM $MgCl_2$ and $(Ca^{2+} + Mg^{2+})$-ATPase activity was assayed as described in Materials and Methods.

tivity was seen with TFP (10–100 μM; Fig. 5) and vanadate (0.1–1.0 mM; Fig. 6). The effect of vanadate was magnesium-dependent as has been described in the red cell [20].

Discussion

The present results demonstrate that plasma membranes purified from a glucose-responsive rat insulinoma exhibit Ca^{2+}-activated Mg^{2+}-dependent ATPase activity. The enzyme was stimulated by calmodulin in calmodulin depleted membranes but not in undepleted membranes suggesting that calmodulin was present in untreated membranes at a concentration sufficient for the full expression of enzyme activity. Calmodulin dependence is a characteristic of the plasma membrane Ca^{2+}-ATP enzyme in other systems (for review, see Ref. 21), while the calcium pump activity of the endoplasmic or sarcoplasmic reticulum is independent of calmodulin [6,16,22,23]. In addition, the calmodulin antagonist trifluoperazine inhibited (Ca^{2+} + Mg^{2+})-ATPase activity in a dose-dependent manner. The exact role of calmodulin in insulin release is unclear. Although calmodulin inhibitors such as trifluoperazine have been shown to inhibit insulin release [24–26] they also interact with other enzymes and inhibit Ca^{2+}-uptake making the interpretation of the release data difficult.

Inhibition of the enzyme was also seen with vanadate, one of the most commonly used inhibitors of the Ca^{2+} pump of plasma membranes. Vanadate increases its inhibitory effect greatly in the presence of Mg^{2+} in the insulinoma similar to findings in the red blood cell [20]. Vanadate, however, is not specific, but inhibits most cation-transport ATPases. Since it inhibits the Ca^{2+}-ATPase from sarcoplasmic reticulum with much lower affinity than the Ca^{2+}-ATPase from plasma membranes, it has been used to differentiate both Ca^{2+}-ATPases. It is interesting that vanadate has been shown to normalize blood glucose concentrations in diabetic rats, however, this seems to be due to an increase in glucose uptake by target tissues and not due to an increase in insulin release [27].

The (Ca^{2+} + Mg^{2+})-ATPase is likely to be involved in the regulation of cytosolic free calcium concentrations in the β-cell. Cytosolic free calcium concentrations rise when β-cells are stimulated with certain secretagogues [3]. It is thought that closing of the ATP-dependent K^+ channel leads to depolarization of the membrane thereby opening voltage-sensitive calcium channels which allow the rapid influx of calcium into the cell [28]. These studies have shown that α-D-glucose, which is a more potent stimulus of insulin release than β-D-glucose [29], has an immediate inhibitory but rapidly reversible effect on the (Ca^{2+} + Mg^{2+})-ATPase. Of all fuels studied, α-D-glucose was the only substance which caused inhibition. Therefore, it is hypothesized that α-D-glucose either binds non-covalently to a site on the enzyme directly or that it binds to a membrane receptor which, via rapid transduction, produces inhibition of the enzyme. The physiological importance of this interaction is manifested by the rapidity, reversibility, stereospecificity and dose-dependence of the glucose effect. The dose dependence is in accordance with the effect of glucose in these cells on insulin release. No stimulated release is seen up to 5 mM glucose, while 30 mM glucose elicits a maximal response [3].

Glyceraldehyde led to almost a tripling in enzyme activity, while no effect on (Ca^{2+} + Mg^{2+})-ATPase activity was seen with other fuel stimuli. This is surprising, since glyceraldehyde leads to a similar increase in insulin release and rise in cytosolic free Ca^{2+} concentration in this insulinoma as glucose does [3]. In addition, α-ketoisocaproate is a much more potent stimulus for insulin release than glucose [13], however, no effect was shown on (Ca^{2+} + Mg^{2+})-ATPase. This suggests that inhibition of the enzyme is not a necessary requirement for insulin release in certain cases. However, further studies are necessary to elucidate the importance of enzyme modification for stimulation of insulin secretion.

It has been suggested that in the red cell glucose inhibits the enzyme via glycosylation of membrane proteins, since in the presence of tunicamycin, an inhibitor of enzymatic protein glycosylation, the effect of glucose is blocked [12]. We also used tunicamycin but did not see any effect on glucose inhibition (data not shown). The rapid reversal of the effect of glucose is also not consistent with covalent modification of membrane proteins by glucose.

In several tissues, it has now been shown that the high-affinity Ca^{2+}-ATPase may not be related to the ATP-dependent Ca^{2+} pump. In liver, the plasma membrane Ca^{2+} pump has a phosphorylated intermediate of M_r 100 000 which is different from that of the Ca^{2+}-ATPase (M_r 140 000) [3]. In corpus luteum plasma membranes, the properties of the Ca^{2+} pump and the phosphorylated intermediate were also quite different from those of the high-affinity Ca^{2+}-ATPase [7]. Discrepancies between both have also been seen in the neutrophil [8] and smooth muscle [6]. With these reservations, we believe that these data provide strong evidence for a direct effect of glucose on calcium-dependent plasma membrane functions. Investigations in plasma membrane vesicles from this insulinoma are necessary to examine whether glucose also has an inhibitory effect on ATP-dependent Ca^{2+} uptake.

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References

1 Herchuelz, A. and Malaisse, W.J. (1978) J. Physiol. (London) 283, 409–424.
2 Herchuelz, A., Couturier, E. and Malaisse, W.J. (1980) Am. J. Physiol. 238, E96–103.
3 Hoenig, M. and Sharp, G.W.G. (1986) Endocrinology 119, 2502–2507.
4 Chan, K.-M. and Junger, K.D. (1983) J. Biol. Chem. 258, 4404–4410.
5 Enyedi, A., Minami, J., Caride, A.J. and Penniston, J.T. (1988) Biochem. J. 252, 215–220.
6 Kwan, C.Y., Kostka, P., Grover, A.K., Lars, J.S. and Daniel, E.E. (1986) Blood Vessels 23, 22–33.
7 Minami, J. and Penniston, J.T. (1987) Biochem. J. 242, 889–894.
8 Ochs, D.L. and Reed, P.W. (1984) J. Biol. Chem. 259, 102–106.
9 Formby, B., Capito, K., Egeberg, J. and Hedeskov, J. (1976) Am. J. Physiol. 230, 441–448.
10 Kotagal, N., Colca, J.R., Buscetto, D. and McDaniel, M.L. (1985) Arch. Biochem. Biophys. 238, 161–169.
11 Levin, S.R., Kasson, B.G. and Driessen, J.F. (1978) J. Clin. Invest. 62, 692–701.
12 Davis, F.B., Davis, P.J., Nat, C., Blas, S.D., MacGillivray, M., Gutman, S. and Feldman, M.J. (1985) Diabetes 34, 639–646.
13 Hoenig, M., Ferguson, D.C. and Matschinsky, F.M. (1984) Diabetes 33, 1–7.
14 Naber, S.P., McDonald, J.M., Jarett, L., McDaniel, M.L., Ludvigsen, C.W. and Lacy, P.E. (1980) Diabetologia 19, 439–444.
15 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
16 Pershadsingh, H.A. and McDonald, J.M. (1980) J. Biol. Chem. 255, 4087–4093.
17 Tsukamoto, Y., Suki, W.N., Liang, T. and Sacktor, B. (1986) J. Biol. Chem. 256, 2718–2724.
18 Pershadsingh, H.A., McDaniel, M.L., Landt, M., Bry, C.G., Lacy, P.E. and McDonald, J.M. (1980) Nature 288, 492–495.
19 Hincke, M.T. and Demaille, J.G. (1984) Biochim. Biochim. Acta 771, 188–194.
20 Schatzmann, H.J., Luterbacher, S., Stieger, J. and Wuthrich, A. (1986) J. Cardiovasc. Pharm. 8 (Suppl. 8), S33–37.
21 Rega, A.V. (1986) in The Ca^{2+} pump of plasma membranes (Rega and Garrahan, eds.), pp. 45–58, CRC Press, Boa Raton.
22 Wibo, M., Morel, N. and Godfraind, T. (1981) Biochim. Biophys. Acta 649, 651–660.
23 Chiesi, M. and Carafoli, E. (1982) J. Biol. Chem. 257, 984–991.
24 Janjic, D., Wollheim, C.B., Siegel, E.G., Krausz, Y. and Sharp, G.W.G. (1981) Diabetes 30, 960–966.
25 Krausz, Y., Wollheim, C.B., Siegel, E. and Sharp, G.W.G. (1980) J. Clin. Invest. 66, 603–607.
26 Valverde, I., Sener, A., Lebrun, P., Herchuelz, A. and Malaisse, W.J. (1981) Endocrinology 108, 1305–1312.
27 Meyerovitch, J., Farfel, Z., Sack, J. and Schechter, Y. (1987) J. Biol. Chem. 262, 6658–6662.
28 Ashcroft, F.M., Kakei, M., Kelly, R.P. and Sutton, R. (1987) FEBS Lett. 215, 9–12.
29 Malaisse, W.J., Malaisse-Lagae, F. and Sener, A. (1983) Physiol. Rev. 63, 773–786.