Glucose stimulates the biosynthesis of a human pancreatic islet cell protein detected by an antiserum against the human erythrocyte glucose transporter

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An antiserum raised against the purified human erythrocyte glucose transporter specifically immunoprecipitated a major protein of M_r 36000 and a minor protein of M_r 58000 from human pancreatic islets. The biosynthetic incorporation of [^{35}S]methionine into the 36-kDa protein increased 2–3-fold in 16 mM D-glucose with 0.1 mM IBMX as compared to islets incubated in 3 mM D-glucose either with or without IBMX. D-Glucose tested without or with IBMX had no effect on the biosynthesis of the 58-kDa protein. The 36-kDa protein was also detected in rat islets and rat islet tumor cells. It is suggested that this protein may represent a D-glucose and cAMP sensitive component of a glucose transport or glucose binding protein in the pancreatic β-cell.

Human erythrocyte glucose transporter *Human pancreatic islet* *Glucose transport* *β-Cell protein* *Glucose regulation* *3-Isobutyl-1-methylxanthine*

1. INTRODUCTION

A prominent function of the pancreatic B-cell is to synthesize and release insulin in response to D-glucose. B-Cells have been found to take up D-glucose by facilitated diffusion utilizing a stereospecific glucose transport system [1]. Experiments with phlorizine, its aglycone phloretin [2–4] and cytochalasin B [5], showed that binding of these compounds to B-cells not only inhibited D-glucose transport, but also affected the release of insulin. This has been interpreted to support the regulator site model for the effect of D-glucose on insulin biosynthesis and release, envisaging that the binding of D-glucose to a hypothetical membrane receptor mediates a signal to the cell [6]. However, substantial experimental evidence favors an alternative hypothesis, the substrate site model [6], in which a signal is generated from the metabolism of D-glucose to act as a trigger for insulin biosyntheses and release. In this model the glucose sensor is the enzyme(s) catalysing a rate-limiting step in glucose metabolism, for instance a kinase catalyzing the phosphorylation of the sugar [7]. A dual-site mechanism in which glucose interaction at the membrane as well as its metabolism within the cell play a role has been suggested to explain available experimental results [8]. The molecular components involved in the uptake and triggering effect of D-glucose are yet to be identified and it remains to be determined whether D-glucose stimulates the biosynthesis of proteins other than (pro)insulin. We have used an antiserum raised against the purified human erythrocyte glucose transporter [9] to determine whether

Abbreviations: NCS, newborn calf serum; HS, human serum; IBMX, 3-isobutyl-1-methylxanthine; FCS, foetal calf serum; HEPES, *N*-hydroxy-ethyl-piperazine–*N*-2-ethane–sulfonic acid; PMSF, phenylmethylsulfonyl-fluoride; PCMBS, *p*-chloromercuribenzenesulfonic acid; SDS, sodium dodecylsulfate; cAMP, cyclic adenosine 3′,5′-monophosphate

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immunologically cross-reactive proteins are present in islet cells. It is tested whether the biosynthesis of such proteins is stimulated by D-glucose alone or combined with the phosphodiesterase inhibitor IBMX which increases the cellular concentration of cAMP in the B-cells and thereby enhances glucose-induced insulin release [10,11].

2. MATERIALS AND METHODS

The antiserum against the human erythrocyte glucose transporter [9] was generously provided by Drs D.C. Sogin and P. Hinkle. Monoclonal antibody against HLA-A,B,C antigens (W6/32) was obtained from Sera Labs. (Sussex). Insulin antibodies against purified porcine insulin were raised in guinea pigs.

Human islets of Langerhans were isolated [12] from the pancreas of 5 cadaver kidney donors, 3 males and 2 females, ranging between 19–36 years of age. Portions of pancreas (~2–3 g) were digested with collagenase. After washing the digest by centrifugation, islets were individually selected under a stereomicroscope. The islets were cultured at 37°C for 1 day in RPMI 1640 supplemented with 10% NCS, transferred to medium in which NCS was replaced by 0.5% HS and finally cultured for 1–7 days. The release of insulin was determined by radioimmunoassay.

Rat islets were isolated from the pancreas of male Wistar rats by collagenase digestion followed by Ficoll (Pharmacia, Uppsala) gradient centrifugation [13].

Cells were prepared from a transplantable rat insulinoma [14] in Hanks balanced salt solution containing 1% bovine serum albumin and separated from erythrocytes by centrifugation (10 min, 500 × *g*) on Ficoll-Paque (Pharmacia).

Human islets were divided into 4 aliquots (150–300 islets each) and transferred to 2 ml L-methionine-deficient RPMI 1640 medium containing 0.5% heat inactivated and dialysed HS, 1 g/l $NaHCO_3$, 100 μg streptomycin/ml, 100 units penicillin/ml, and 0.5 mCi [^{35}S]methionine (>900 Ci/mmol, New England Nuclear). D-Glucose was either 3 mM or 16 mM and the incubations were carried out for 20 h either in the absence or presence of 0.1 mM IBMX. Rat islets and rat insulinoma cells were labelled for 20 h and 6 h, respectively, in the same type of medium at 16 mM glucose without IBMX and substituting HS with 2.5% FCS. In one experiment human islets were labelled with [^{35}S]cysteine (>600 Ci/mmol, Radiochemical Centre, Amersham) in addition to [^{35}S]methionine in order to obtain radioactive labelling of human proinsulin and insulin.

After labelling, islets or cells were washed twice by centrifugation (islets, 30 s 150 × *g*; cells, 5 min 50 × *g*) in medium containing 5 mM L-methionine and once in 10 mM Hepes (pH 7.4) containing 150 mM NaCl, 5 mM methionine and 2 mM PMSF and then lysed in the same buffer containing 2000 KIE/ml trasylol, 0.1 mM PCMBS, 5 mM EDTA, 5 mM iodoacetamide and 1% NP-40. Islets were frozen and thawed once and then kept on ice for an additional 30 min before the lysate was centrifuged for 30 min at 100000 × *g*. The supernatant was incubated at 4°C for 1 h with 10 μl normal rabbit serum/100 μl supernatant, followed by adsorption to an excess of protein A–Sepharose CL-4B (Pharmacia) before immunoprecipitation with immune serum. Aliquots (100 μl, 1–2 × 10^7 cpm) of pre-absorbed lysate were incubated with 20 μl immune serum or normal serum for 16 h followed by incubation for 30 min at 4°C with 100 μl pre-swollen protein A–Sepharose. The protein A–Sepharose antibody–antigen complex was washed 5 times by centrifugation in 10 mM Hepes buffer containing 150 mM NaCl, 2 mM PMSF and 0.5% NP-40 (pH 7.4) and once in cold re-distilled water. Immune complexes were analysed by SDS–polyacrylamide gel electrophoresis on 7.5–15% gradient or 10% uniform slab gels and processed for autoradiography [15].

Autoradiograms were scanned on an LKB Ultro Scan Laser Densitometer using a [^{14}C]methylated protein kit (Radiochemical Centre, Amersham) as standard to corroborate an approximate proportionality of amount of radioactivity to band intensity.

3. RESULTS

The antiserum to the human erythrocyte glucose transporter specifically immunoprecipitated a major band at M_r 36000 from human islets (fig.1a), rat islets (fig.1c) and rat insulinoma cells (fig.1e). A faint band at M_r 58000 (fig.2A,C,E,G; fig.1c)

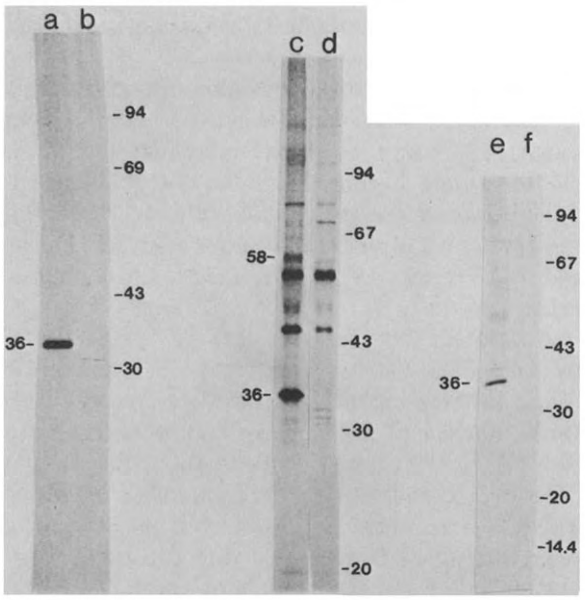

Fig.1. SDS–polyacrylamide gel electrophoresis and autoradiography of immunoprecipitates of human islet (a,b), rat islet (c,d) and rat islet tumor cell (e,f) proteins with antiserum against human erythrocyte glucose transporter (a,c,e) and normal rabbit serum (b,d,f). Positions of M_r markers are shown to the right.

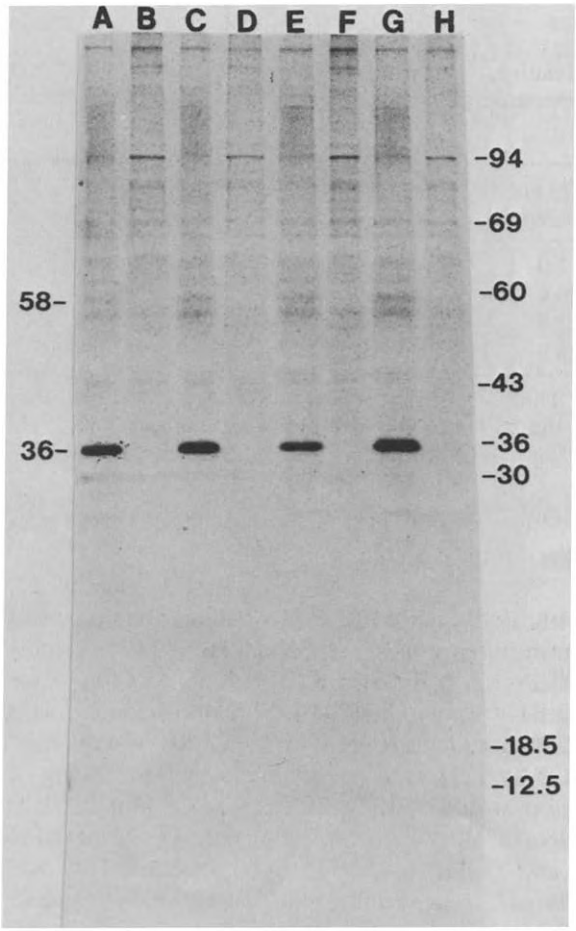

Fig.2. SDS–polyacrylamide gel electrophoresis and autoradiography of immunoprecipitates of human islet cell proteins with antiserum against human erythrocyte glucose transporter (A,C,E,G) and normal rabbit sera (B,D,F,H). Islets were biosynthetically labelled for 20 h with [^{35}S]methionine in the presence of 3 mM glucose (A,B), 16 mM glucose (C,D), 3 mM glucose + 0.1 mM IBMX (E,F) and 16 mM glucose + 0.1 mM IBMX (G,H). Positions of M_r markers are shown to the right.

was immunoprecipitated from human islets as well as from rat islets but not from rat insulinoma cells (fig.1e). Normal rabbit serum did not immunoprecipitate those bands (fig.1b,d,f; fig.2B,D,F,H).

Densitometric analyses of autoradiograms similar to that in fig.2 demonstrated that the biosynthesis of the 36-kDa protein was increased after incubation with 16 mM D-glucose and 0.1 mM IBMX as compared to 3 mM D-glucose with ($p < 0.01$) or without IBMX ($p < 0.001$) (table 1). There was no effect of 0.1 mM IBMX at 3 mM D-glucose (table 1). In the absence of IBMX, the effect of 16 mM D-glucose varied between islets isolated from the 5 different donors, the individual values of the relative density of the 36-kDa band was 0.8, 0.8, 1.2, 1.8 and 2.6, respectively. No difference was found in the density of the faint 58-kDa protein band.

In lysates of human islets labelled with [^{35}S]methionine for 20 h, the total trichloroacetic acid-precipitable counts were increased by 17% and 22%, respectively, in islets incubated without or with 0.1 mM IBMX at 16 mM glucose as compared to islets incubated at 3 mM glucose. The effect of glucose and IBMX on class I major histocompatibility antigens (HLA-A,B,C) [15] biosynthesis was also investigated and compared to that of proinsulin and insulin. HLA-A,B,C antigens were immunoprecipitated by the monoclonal antibody W6/32 and (pro)insulin was immunoprecipitated by a guinea pig antiserum against porcine insulin from human islets labelled for 20 h in the presence of 3 mM or 16 mM D-glucose with or

Table 1

Relative amounts of the 36-kDa antigen immunoprecipitated by anti-erythrocyte glucose transporter

D-Glucose (mM)	IBMX (mM)	n	Relative amount
3.0	0	5	1
16.0	0	5	1.4 ± 0.9
3.0	0.1	5	1.1 ± 0.4
16.0	0.1	5	2.4 ± 0.2[a]

[a] The effect of D-glucose with IBMX was different from that at 3 mM D-glucose both without ($p < 0.001$) and with IBMX ($p < 0.01$) (paired Student's t-test)

Results are shown as mean ± SD for the no. expt. (n)

without 0.1 mM IBMX. Densitometric analyses of autoradiograms showed no effect of D-glucose whether IBMX was present or not, on the biosynthesis of either the 44-kDa heavy chain or the 12.5-kDa β_2-microglobulin light chain of the HLA-A,B,C antigens. The autoradiographic density of the proinsulin band was increased about 4-fold in islets labelled at 16 mM D-glucose as compared to 3 mM D-glucose. IBMX slightly enhanced the density of the proinsulin band at 16 mM D-glucose. The broad appearance of the band corresponding to insulin did not permit accurate densitometric analyses. The release of insulin during 20 h incubation was 0.4 and 1.04 ng/islet at 3.0 mM D-glucose without and with 0.1 mM IBMX, respectively. At 16 mM D-glucose the corresponding values were 2.69 and 3.01 ng/islet, respectively.

4. DISCUSSION

Antisera against the purified glucose transporter of human erythrocytes, which detects an M_r 55000 protein [9], have been used tentatively to identify a glucose transport protein of M_r 41000 in chicken embryo fibroblasts [17], a protein of M_r 45000 in rat adipocytes [18,19] and a protein of M_r 55000 in HeLa cells [9]. Photoaffinity labelling of the glucose transporter with [^{3}H]cytochalasin B confirmed that the transport protein has an M_r of 45000–46000 in rat adipocytes [20,21] and chicken embryo fibroblasts [22], but an additional protein component of M_r 50000–52000 was also labelled [20,22].

In the present studies with human and rat islets it was found that the glucose transporter antiserum specifically immunoprecipitated a major band at M_r 36000 and a minor band at M_r 58000. In rat islet tumor cells the only protein specifically precipitated had an M_r of 36000 which indicates that this protein is present in insulin producing B-cells.

Although the reactivity with the glucose transporter antisera suggest that one or both of these proteins share antigenic determinants with the human erythrocyte glucose-transport protein in islet cell the possibility of a cross-reacting protein unrelated to the transporter cannot be ruled out. The M_r determined for those proteins is different from the M_r determined for glucose transporter molecules in other cells. It is important to note that experiments with the same antiserum used here have detected components of different relative molecular sizes in different cells [9,18]. We speculate therefore that glucose transport proteins in different cells may not have the same relative molecular sizes but share similar antigenic determinants. It will be important to determine the M_r of islet B-cell components which can be photoaffinity labelled with radioactive cytochalasin B. Such studies are difficult to carry out with human islets due to the lack of sufficient material to prepare membranes but are in progress with the rat islet tumor cells.

The stimulating effect of glucose combined with IBMX on the biosynthetic labelling of the 36-kDa protein suggest that this protein is somehow regulated by glucose and islet cAMP levels. It is also possible that the increased insulin release promotes the biosynthesis due to an increased membrane turnover during exocytosis. Recent studies with islet cell surface antibodies showed that IBMX increased the internalization of islet cell surface-bound antibodies [23].

We also found that glucose and IBMX enhanced the biosynthetic incorporation of [^{35}S]cysteine into human proinsulin. However, no effect of glucose and/or IBMX was detected on the biosynthesis of the heavy and light chain of the class I major histocompatibility antigens. Although total islet protein synthesis was stimulated by glucose these data indicate that the effect of D-glucose combined

with IBMX does not affect all islet proteins and that the biosynthesis of the 36-kDa protein is specifically stimulated by D-glucose.

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