Diabetes Affects Similarly the Catalytic Subunit and Putative Glucose-6-phosphate Translocase of Glucose-6-phosphatase*

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The effect of streptozocin diabetes on the expression of the catalytic subunit (p36) and the putative glucose-6-phosphate translocase (p46) of the glucose-6-phosphatase system (G6Pase) was investigated in rats. In addition to the documented effect of diabetes to increase p36 mRNA and protein in the liver and kidney, a ~2-fold increase in the mRNA abundance of p46 was found in liver, kidney, and intestine, and a similar increase was found in the p46 protein level in liver. In HepG2 cells, glucose caused a dose-dependent (1–25 mM) increase (up to 5-fold) in p36 and p46 mRNA and a lesser increase in p46 protein, whereas insulin (1 μM) suppressed p36 mRNA, reduced p46 mRNA level by half, and decreased p46 protein by about 33%. Cyclic AMP (100 μM) increased p36 and p46 mRNA by >2- and 1.5-fold, respectively, but not p46 protein. These data suggest that insulin deficiency and hyperglycemia might each be responsible for up-regulation of G6Pase in diabetes. It is concluded that enhanced hepatic glucose output in insulin-dependent diabetes probably involves dysregulation of both the catalytic subunit and the putative glucose-6-phosphate translocase of the liver G6Pase system.

A number of previously reported glucose-6-phosphatase (EC 3.1.3.9) activity changes has been shown to be associated with long term regulation in the 36-kDa catalytic subunit (p36) mRNA level. Insulin deficiency achieved by acute fasting or experimental diabetes in the rat increases G6Pase activity (1) and p36 mRNA (2) regardless of glycemia (3, 4). Studies in Faoo hepatoma cells showed that insulin suppressed p36 mRNA levels and that cyclic AMP (cAMP) counteracted this effect (5), whereas cAMP alone increased p36 mRNA by a factor of four (6). Liu et al. (2) observed that blood glucose concentrations correlated with G6Pase mRNA levels, suggesting that G6Pase transcription might be controlled by glucose or a glucose-derived metabolite. This presumption was later verified by direct demonstration of glucose (7) and fructose 2,6-bisphosphate (8) stimulation of p36 expression.

A major breakthrough in the G6Pase field has been the finding of a human cDNA sequence encoding a 46-kDa protein (p46) homologous to bacterial phosphate ester transporters (9). Mutations of the p46 gene were found in glycogen storage disease type 1b patients, which have impaired G6Pase activity despite normal p36 catalytic subunit (9). It was therefore proposed that p46 was the putative G6P translocase of the G6Pase system. Until now, there has been no report on the regulation of the expression of p46, and its role in the control of G6Pase activity is unknown. Here we show that p46 mRNA and protein are induced by glucose and are repressed by insulin, in parallel to p36, and that p36 and p46 mRNA are increased by cAMP. These results may explain the present finding that both the catalytic subunit and the putative G6P translocase are impaired in insulin-dependent diabetes.

EXPERIMENTAL PROCEDURES

Animals—Male Wistar rats (200–250 g) (Charles River, Canada) were given one intravenous administration of streptozocin (75 mg/kg) (Upjohn, Ontario) dissolved in citrate, pH 4.0 (n, 6) or vehicle alone (n, 7). The diabetic state was assessed by the presence of hyperglycemia after a 24-hour fasting period by glucosuria and by a lesser weight gain. The two groups were sacrificed by decapitation after an overnight fast, 1 week after the treatment. Blood was obtained as well as tissue samples (liver, heart, hind limb skeletal muscle, kidney, small intestine, and brain), that were quickly freeze-clamped in liquid nitrogen and kept at −80 °C for subsequent analysis.

Preparation of Rat Liver Microsomes and G6Pase Assay—Rat liver microsomes were isolated individually from each rat as described previously (10) and resuspended at a protein concentration of about 7 mg/ml in 50 mM Tris-Hepes, pH 7.3, 250 mM sucrose. G6Pase activity was assayed with 5 mM [U-14C]G6P after detergent (CHAPS) 0.8% treatment as described in Ref. 10.

Cell Culture—HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% bovine fetal serum. To investigate the effect of glucose, cells were kept in the presence of the indicated glucose concentrations for 48 h at 37 °C. Insulin (1 μM) or cAMP (0.5–100 μM in the presence of 50 μM methylisobutylxanthine (MIX) to inhibit cAMP phosphodiesterases) was added to 80–90% confluent HepG2 cells in serum-free Dulbecco’s modified Eagle’s medium for the indicated times, in the presence of 5 mM glucose. Cells were harvested by scraping after different times of glucose or hormone treatment and kept at −80 °C for subsequent analysis.

Immunoblotting Analysis—The rabbit polyclonal antiserum against the recombinant G6Pase catalytic subunit (antibody against p36) was a kind gift from Dr. J. J. Chou (National Institutes of Health, Bethesda, MD). The rabbit antiserum against the N terminus of p46 (GYGYYRTVIFSAMFGGY) was produced in our laboratory as explained by Xie et al. (11) and according to the methods described in detail by Mechin et al. (12). Micromolar concentrations of mutants or residues of different tissues or HepG2 cell homogenates (50 μg protein) were subjected to a 12% SDS-polyacrylamide gel electrophoresis at 100 V for about 1 h in Laemmli buffer (13). Proteins were then electrotransferred to a nitrocellulose membrane at 100 V for 1 h. The membrane was saturated for 1 h in 100 ml Tris-buffered saline, pH 7.5, containing 10% dried milk (w/v) and further incubated overnight in the primary antibody solution (diluted to 1/500 for anti-p36 and 1/2000 for anti-p46). After washing, the membrane was incubated in the alkaline phosphatase-conjugated anti-rabbit IgG solution (diluted to 1/2000) for 2 h, washed again, and detected in 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate system (Promega). The membrane was scanned, and the

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1 The abbreviations and trivial names used are: p36, catalytic subunit of G6Pase; G6Pase, glucose-6-phosphatase; G6P, glucose-6-phosphate; p46, putative G6P translocase; MIX, methylisobutylxanthine; MOPS, 4-morpholinepropanesulfonic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonic acid.

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Glucose-6-Phosphatase Components in Diabetes

TABLE I
Characteristics of control and diabetic rats

| Group    | n | Weight difference*  | Plasma glucose | Glucosuria | G6Pase activity |
|----------|---|---------------------|----------------|------------|-----------------|
|          |   | g                   | mEq/g protein  | positive   | 98 ± 8          |
| Control  | 7 | 16.1 ± 3.5          | 3.4 ± 0.08     | negative   | 108 ± 19        |
| Diabetic | 6 | -7.2 ± 8.0          | 20.1 ± 3.3     | positive   | 108 ± 19        |

* This is the weight difference from treatment to sacrifice.

RESULTS AND DISCUSSION

Characteristics of Diabetic Rats—Table I shows the effect of streptozocin or vehicle treatment on body weight, fasting blood glucose, glucosuria, and liver G6Pase activity of the two groups of rats. As expected, diabetic animals were markedly hyperglycemic, had glucosuria, and gained less weight after treatment compared with the corresponding controls. G6Pase activity measured in microsomes isolated from livers of the rats was increased by about 2-fold in the diabetic group, as documented before (1, 2).

Effect of Streptozocin Diabetes on p36 and p46 mRNA and Protein in Different Tissues—The catalytic subunit of G6Pase, p36, is known to be expressed mainly in glucogenic tissues such as liver and kidney (16) and has been also found recently in small intestine (17). It is apparent from the Northern blots shown in Fig. 1A that the tissue distribution of p46 in rats is parallel to that of p36 (16, 17) with higher mRNA abundance of p46 in liver and kidney compared with intestine and no signal in skeletal muscle as well as in brain and heart (results not shown). Using a more sensitive Northern blotting analysis with poly(A)* RNAs, Lin et al. (18) found p46 mRNA in almost all rat tissues investigated.

Insulin-deficient streptozocin diabetes increased the p46 transcript by 2–3-fold in liver, kidney, and intestine and, as reported previously, also increased liver p36 mRNA (Fig. 1A) (2). Fig. 1B further shows by immunoblotting analysis that p36 protein is increased in diabetic liver and kidney compared with control as shown before (2, 16) and that p46 protein is increased in liver in parallel with p36. Both proteins were undetectable either in control or in diabetic skeletal muscle. These results indicate that in the G6Pase system, p46, in addition to p36, could play a role in glucose production by liver and their dysregulation in diabetes. They also show that p46 mRNA is up-regulated similarly to p36 mRNA by diabetes in tissues (liver, kidney, and intestine) where p36 is present and where p36 mRNA is also enhanced (2, 17, 19).

Effect of Glucose, Insulin, and cAMP on the mRNA and Protein Levels of p36 and p46 in HepG2 Cells—The relative contributions in diabetes of insulin deficiency, hyperglycemia, and cAMP (resulting from counterregulatory hormones unopposed by the lack of insulin) on p36 and p46 were further assessed in HepG2 cells. A dose-dependent increase by glucose (1–25 mM) of both p36 and p46 mRNA was observed in these cells sampled from control and diabetic rats as described under “Experimental Procedures.” B, immunoblotting analysis of p36 and p46 in microsomal fractions isolated from the indicated tissues as described under “Experimental Procedures.” Analysis was performed several times for all tissues with similar results. A representative experiment is shown.
Taken together, these results indicate that several parameters (insulin deficiency, hyperglycemia, and increased intracellular cAMP consequent to unopposed counterregulatory hormones) contribute independently from each other to elevated p36 and p46 in insulin-dependent diabetes and may exacerbate hyperglycemia by increased hepatic glucose output. Consistent with this idea, a potent inhibitor of p46, chlorogenic acid (22), is currently being studied as a pharmaceutical tool to inhibit hepatic glucose production in diabetes (23). Overexpression of p36 in primary hepatocytes with a recombinant adenovirus (24) resulted in increased flux through G6Pase, suggesting that an increase in the catalytic subunit alone is sufficient to activate G6Pase in the intact cell. The mechanism(s) by which overexpression of p46 might affect p36 catalytic activity is however not known and deserves further studies.

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