Platelet-derived Growth Factor Regulates Glucose Transporter Expression*

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Regulated glucose transport is necessary for controlled cellular proliferation. In this communication, we demonstrate that a growth factor, namely platelet-derived growth factor (PDGF), regulates expression of the glucose transporter gene. PDGF induced a 1.7-fold increase in both the rate of glucose transport and the amount of membrane-associated glucose transporter protein in mouse fibroblasts 6 h after treatment. This was accompanied by a 6-fold increase in the accumulation of the HepG-2/rat brain glucose transporter mRNA. PDGF induced both an increase in the rate of transcription of the glucose transporter gene and an increase in the stability of its mRNA. This induction could be achieved in the presence of cycloheximide. The glucose transporter is therefore a member of the class of PDGF-inducible genes known as competence genes.

Cellular proliferation requires glucose metabolism, and controlled proliferation requires that this metabolism be homeostatically regulated. Regulatory control can be exerted at several levels, beginning with glucose entry into cells which occurs via carrier-mediated facilitated diffusion (1). One such glucose carrier is the glucose transporter, for which highly homologous cDNAs have been cloned from the human HepG-2 hepatoblastoma cell line (2) and from rat brain (3). These cDNAs have been used to analyze transporter expression under a variety of conditions. For example, oncongenically transformed cells accelerate glucose uptake (4, 5), and elevated levels of glucose transporter protein can accompany this phenotype in some transformed cells (6). The transporter cDNA probes have been used to demonstrate that elevated glucose transport in cells transformed by the v-src and c-ras genes.

Regulated glucose transport is necessary for controlled cellular proliferation. In this communication, we demonstrate that a growth factor, namely platelet-derived growth factor (PDGF), regulates expression of the glucose transporter gene. PDGF induced a 1.7-fold increase in both the rate of glucose transport and the amount of membrane-associated glucose transporter protein in mouse fibroblasts 6 h after treatment. This was accompanied by a 6-fold increase in the accumulation of the HepG-2/rat brain glucose transporter mRNA. PDGF induced both an increase in the rate of transcription of the glucose transporter gene and an increase in the stability of its mRNA. This induction could be achieved in the presence of cycloheximide. The glucose transporter is therefore a member of the class of PDGF-inducible genes known as competence genes.

Experimental Procedures

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Experimental Procedures

Cell Culture and Growth Factors—Balb/c 3T3 cells (clone A31) were routinely grown in Dulbecco’s modified Eagle’s medium (DME medium) supplemented with 10% heat-activated bovine calf serum and antibiotics. Confluent density-arrested monolayer cell cultures were prepared as previously described (20) and then transferred to fresh DME medium supplemented with 0.5% platelet-poor plasma (PPP) (21). Cells were kept in 0.5% PPP for 48 h prior to analysis. Pure PDGF (recombinant v-src; B-chain homodimer) and IGF-I were obtained from Amgen, Thousand Oaks, CA; porcine insulin was obtained from Lilly.

2-Deoxyglucose Uptake—Cells were prepared as described above, washed in phosphate-buffered saline, and incubated at 37 °C in glucose-free DME medium supplemented with 0.1% fatty acid-free bovine serum albumin and 100 nM 2-deoxyglucose. After 5 min, 2 × 10° cpm [H]2-deoxyglucose (specific activity 5 mCi/mmol, Du Pont-New England Nuclear) was added. After 5 min, uptake was stopped by adding cold phosphate-buffered saline with 0.3 mM phloretin and was measured as nanomoles of 2-deoxyglucose/μg of DNA/5 min (7). Induced transport is reported as fold induction over the control ± S.E.

Immunoblotting—Cells were washed twice in cold phosphate-buffered saline, scraped from plates, and centrifuged at 500 × g for 5 min. They were then solubilized in TES-PI (20 mM Tris-HCl, 1 mM EDTA, 0.25 M sucrose, and 5 μg/ml each aprotinin, leupeptin, and pepstatin A), and membranes were isolated by centrifugation at 95,000 rpm for 20 min at 4 °C in a Beckman TL100 centrifuge. The pellet was solubilized in TES, and 50 μg of protein (as determined by Bradford assay) was subjected to electrophoresis in a 10% polyacrylamide gel in SDS. Separated proteins were transferred to nitrocellulose filters. Immunodetection was performed using an antisum raised against a peptide consisting of 16 amino acids from the C-terminal portion of the HepG-2 glucose transporter kindly supplied by Dr. Bernard Thorens, Whitehead Institute, Boston, MA. Bound antibody was detected by using 125I-Protein A.

RNA Analysis—Cells were scraped directly into a solution of 4 M guanidine isothiocyanate, 25 mM sodium citrate (pH 7.0), 100 mM 2-mercaptoethanol, and the RNA purified by centrifugation through a cushion of 5.7 M CsCl followed by ethanol precipitation (22). Twenty

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micrograms of purified RNA were electrophoresed through a 1.5% agarose, 2.2 M formaldehyde gel and transferred to nitrocellulose filters in 20 × SSC. When using DNA probes, baked filters were prehybridized and hybridized at 42 °C in 1× SSC solutions described in Ref. 7. For RNA probes, filters were prehybridized and hybridized at 42 °C in a solution consisting of 50% formamide, 5 × SSC, 150 μg/ml salmon sperm DNA, 50 mM Tris-HCl (pH 7.5), 0.025% sodium pyrophosphate, 1% SDS, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 5 mM EDTA, 0.2% bovine serum albumin. Washes were 2 × SSC at 65 °C (two washes at 15 min each) followed by 0.1 × SSC at 65 °C (two washes at 15 min each). DNA probes were labeled using the Klenow fragment of Escherichia coli DNA polymerase I and random priming to a specific activity of greater than 10⁶ cpm/μg. RNA probes were labeled using T7 RNA polymerase (Promega, Madison, WI) and [α-32P]UTP with the appropriate DNA template. The template was removed using RQ DNase (Promega) and the RNA purified on an Ektacyt R column (Schleicher and Schuell). Probes were as follows: glucose transporter, for nick translation, a mixture of the 450-base pair pGT25S and 2400-base pair pGT25L EcoRI fragments, and for RNA probes, the nearly full-length pGT (2); actin, a 600-base pair PstI fragment of mouse β-actin CDNA, a gift of Dr. B. Spiegelman, Dana-Farber Cancer Institute (20). Induced RNA accumulation is described as -fold induction over the control ± S.E.

Nuclear Run-on Transcriptional Analysis—Run-on analysis was performed as described (24). Briefly, after the appropriate treatment, cells (four 150-cm² plates of confluent cells) were scrapped into cold phosphate-buffered saline. Nuclei were isolated by Nondenat P-40 lysis and stored at −70 °C in a final volume of 200 μl in glycerol buffer. To radiolabel nascent RNA, the nuclei were thawed in the presence of an equal volume of 2 × transcription buffer (17) containing rNTPs (including 2.5 μM unlabeled UTP) and 200 μCi of [α-32P]UTP (600 Ci/mmol; Du Pont-New England Nuclear). Run-on transcription was allowed to proceed at 27 °C for 30 min. Nuclei were pelleted at 200 × g and resuspended in 400 μl of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 100 mM NaCl, 20 mM MgCl₂ with 50 μg of tRNA and 3 μl of RNasin (Promega). DNA was digested with 15 μl of RQ DNase (Promega). This was followed by a protease K digest (Bethesda Research Laboratories) and extraction with phenol, phenol:chloroform, and chloroform. The RNA was precipitated with ethanol twice. Equal amounts of radioactive RNA were hybridized to DNA immobilized on nitrocellulose filters in 10 mM TES (pH 7.4) 0.2% SDS, 10 mM EDTA, 300 mM NaCl at 65 °C for 36 h. Addition of equal amounts of radioactive RNA was confirmed by hybridization to 100 ng of BALB/c liver DNA. Filters were washed in 2 × SSC at 65 °C for 2 h and then digested with 10 μg/ml RNase A (Sigma) in 2 × SSC at 37 °C for 30 min. Target DNA sequences (5 μg each) were denatured in 0.1 M NaOH before blotting.

Densitometric Analysis—Densitometric measurements were performed using an LKB Ultrascan enhanced laser densitometer (Pharmacia LKB Biotechnology Inc.). Integrations were performed using software provided by LKB.

RESULTS

Purified Serum Growth Factors Induce Glucose Transporter Expression—BALB/c 3T3 fibroblasts were grown to confluence and growth arrest in tissue culture. The cells were deprived of growth factors by placing them in medium supplemented with 0.5% PPP for 48 h. Six hours after exposing the cells to fresh medium containing 10% calf serum, glucose transport was induced 2.0-fold (average of two experiments, data not shown). These experiments used whole serum, which contains two categories of growth factors. Competence factors (such as PDGF) act on resting cells to make them responsive to progression factors (such as the IGFs, insulin, or epidermal growth factor) (20). The combination of competence and progression factors is required for optimal cellular proliferation. Purified factors of both types were tested for their ability to induce glucose transporter expression. While not the focus of this report, both IGF-I and insulin were able to increase glucose transporter 2–4-fold with a peak effect 6 h after treatment (data not shown). Insulin accomplished this at concentrations at which only the insulin receptor should be occupied (50 ng/ml) and at which both the IGF and insulin receptors should be occupied (1000 ng/ml).

Recombinant PDGF increased glucose transporter 1.7 ± 0.06-fold (average ± S.E. of four independent experiments, each taking the average of three determinations) in 3T3 cells 6 h after treatment (Fig. 1 shows the mean of two such experiments). PDGF was able to increase the levels of glucose transporter protein 1.7-fold (average of two experiments) as determined by immunoblotting (Fig. 2A). Thus PDGF appeared to accelerate glucose transport in 3T3 cells, at least in part, by inducing accumulation of the glucose transporter protein.

PDGF Directly Induces Glucose Transporter Expression—We examined PDGF-induced glucose transporter expression to test whether the induction of this gene would be similar to that of other PDGF- inducible genes. Fig. 2B shows that the increased amount of transporter protein induced in response to PDGF is accompanied by an increase in the abundance of the glucose transporter mRNA. There was a low level of constitutive expression of transporter mRNA in the absence of PDGF. Four to six hours after PDGF treatment, the accumulation of transporter mRNA rose 5.1 ± 0.9-fold (the average ± S.E. from six independent experiments) and decreased to basal levels 18–24 h later. Fig. 2C shows that PDGF induced glucose transporter mRNA expression to similar levels in the presence of cycloheximide. The reduction in actin mRNA levels seen after cycloheximide treatment may be due to the harsh pretreatment conditions used in these experiments. Ordinarily, the long half-life and high abundance of actin mRNA (28) precludes any decrease in its abundance when cells are made quiescent in 5% PPP (for example, see Refs. 17 and 24). Keeping 3T3 cells in 0.5% PPP for 48 h may have allowed actin mRNA levels to decrease enough that subsequent cycloheximide treatment led to a detectable further decrease in abundance. Regardless of mechanism, the long half-life of actin mRNA still makes normalization of a short-lived mRNA, such as that for the glucose transporter (see below), to actin mRNA levels valid and only strengthens the argument that PDGF induces transporter expression in the presence of cycloheximide. Thus PDGF induces transporter expression directly, i.e. no newly synthesized intermediate protein is required. Cycloheximide alone induced a small increase in transporter mRNA.

PDGF Induces Both Transcription and Stabilization of the Glucose Transporter mRNA—PDGF might increase transporter mRNA levels by increasing the rate of transporter mRNA transcription, by decreasing the rate of transporter mRNA degradation, or by a combination of these mechanisms. The nuclear run-on transcriptional analysis of Fig. 3 shows that neither of these events occurred. At 6 h, after PDGF addition, there was no increase in transcription of transporter mRNA (data not shown). The reduction in actin mRNA levels seen after cycloheximide treatment may be due to the harsh pretreatment conditions used in these experiments. Ordinarily, the long half-life and high abundance of actin mRNA (28) precludes any decrease in its abundance when cells are made quiescent in 5% PPP (for example, see Refs. 17 and 24). Keeping 3T3 cells in 0.5% PPP for 48 h may have allowed actin mRNA levels to decrease enough that subsequent cycloheximide treatment led to a detectable further decrease in abundance. Regardless of mechanism, the long half-life of actin mRNA still makes normalization of a short-lived mRNA, such as that for the glucose transporter (see below), to actin mRNA levels valid and only strengthens the argument that PDGF induces transporter expression in the presence of cycloheximide. Thus PDGF induces transporter expression directly, i.e. no newly synthesized intermediate protein is required. Cycloheximide alone induced a small increase in transporter mRNA.

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FIG. 1. Confluent density-arrested cultures of 3T3 cells were kept in DME medium supplemented with 0.5% PPP for 48 h. Recombinant PDGF (v-sis) was added to a final concentration of 10 ng/ml. At the indicated times, glucose transport was determined in triplicate and the average taken. The figure shows the average and range of two such independent experiments.
PDGF and Glucose Transport

A. 0 4 6 8

B. 0 2 4 6 12 18 24

C. NA  PDGF  CH  PDGF/CH

GT  Actin

Fig. 2. A, confluent density-arrested cultures of 3T3 cells were kept in DME medium supplemented with 0.5% PPP for 48 h. Recombinant PDGF (v-sis) was added to a final concentration of 15 ng/ml. (This particular lot of v-sis had a slightly lower specific activity, measured as mitogenic activity, than other lots used in this study. Amounts of v-sis added to cells were normalized to a concentration that induced 100% \(^[^3]H\)thymidine uptake in 3T3 cells.) Cells were collected at the indicated times after PDGF treatment and prepared for immunoblotting and glucose transporter detection as described under "Experimental Procedures." B, cells prepared as in A were treated with 10 ng/ml PDGF. At the indicated times (in hours), RNA was collected, and 20 \(\mu\)g of total RNA in each lane was analyzed by Northern blotting using the glucose transporter cDNA as probe. (Some blots, such as this one, were probed using nick-translated cDNA and others with labeled RNA. Both methods yielded similar results.) C, cells were prepared as described in A. Six hours after the indicated treatment, RNA was collected from the cells, and 20 \(\mu\)g/lane was analyzed by Northern blotting. The same blot was probed for glucose transporter (GT) and actin mRNAs. NA, no addition; PDGF, 10 ng/ml PDGF; CH, 50 \(\mu\)g/ml cycloheximide; PDGF/CH, 10 ng/ml PDGF with 50 \(\mu\)g/ml cycloheximide.

shows that PDGF induced glucose transporter mRNA transcription. The induction was only 3–4-fold, slightly less than the accumulation of transporter mRNA in parallel experiments. An increase in transporter mRNA half-life could account for the remainder of the mRNA accumulation. Fig. 4 shows that PDGF increased the stability of the glucose transporter mRNA. Cells were treated with actinomycin D either without or 6 h after PDGF treatment, and RNA was analyzed at various times after addition of the inhibitor. Fig. 4 shows that in the absence of PDGF, the transporter mRNA had a mean half-life of 47 min. Six hours after PDGF treatment, this half-life was extended to 160 min. Thus PDGF increased the levels of glucose transporter mRNA by a combination of transcriptional induction and post-transcriptional stabilization.

DISCUSSION

PDGF exerts its mitogenic effect on fibroblasts, in part, by inducing the expression of genes whose products are required for cellular proliferation (14–19, 25). For example, the PDGF-inducible proto-oncogenes c-myc and c-fos have been shown to act as intracellular mediators of the growth response to PDGF (26–33). In this communication, we have demonstrated that the glucose transporter defined by the HepG-2/rat brain transporter cDNA is also a PDGF-inducible gene. PDGF
induces the expression of the transporter gene by increasing
the rate at which the gene is transcribed and by decreasing
the rate at which its mRNA is degraded. Induction of trans-
porter mRNA in the presence of cycloheximide shows that its
expression is not simply a result of progression through the
cell cycle, since cycloheximide prevents such progression.
Thus by definition, this particular glucose transporter gene is
a competence gene (14). Interestingly, the degree of mRNA
induction by PDGF in several experiments (5-fold) was never
matched by the degree of transport or transporter protein
induction (up to 2-fold). This discordance between mRNA
and protein function suggests the presence of some translational
or post-translational control over glucose transporter
function, as suggested by others (34–36). We are also inves-
tigating this possibility.

Unlike other competence genes (such as c-myc, c-fos, JE,
and KC), the glucose transporter is induced as well by the
progression factors, IGF-I and insulin (data not shown), as
by the competence factor, PDGF. One class of IGF-I-inducible
progression factors, IGF-I and insulin (data not shown), as
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and deregulated expression of the glucose transporter in cells
receptors of the tyrosine kinase class regulate transporter
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An interesting question for further study is whether the
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The regulation of cell proliferation by growth factor-in-
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