Insulin stimulates glyceraldehyde-3-phosphate dehydrogenase gene expression through cis-acting DNA sequences

(transcription/DNA transfer)

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ABSTRACT Glyceraldehyde-3-phosphate dehydrogenase [GAPDH; D-glyceraldehyde-3-phosphate:NAD$^+$ oxidoreductase (phosphorylating), EC 1.2.1.12] mRNA levels are induced by physiologic concentrations of insulin in cultured 3T3-F442A adipocyte and H35 hepatoma cell lines. To examine the mechanism by which insulin regulates GAPDH mRNA levels in these two insulin-sensitive tissues, we have isolated a functional human GAPDH gene. When stably transfected and expressed in 3T3-F442A preadipocytes and H35 hepatoma cells, the intact human GAPDH gene is induced 10-fold by insulin in 3T3-F442A adipocytes and 3-fold by insulin in H35 hepatoma lines, which is similar to the induction obtained with the endogenous gene. A human GAPDH–chloramphenicol acetyltransferase construct, containing sequences −487 to +20 of the human gene fused to the chloramphenicol acetyltransferase gene, is regulated by insulin in stably transfected 3T3 adipocytes and stably or transiently transfected H35 hepatoma cell lines, whereas the Rous sarcoma virus–chloramphenicol acetyltransferase fusion protein is not. Thus, the inductive effect of insulin on human GAPDH gene expression is mediated through cis-acting sequences located between −487 and +20 of the human GAPDH gene.

The interaction of insulin with its cell surface receptor-associated tyrosine kinase initiates diverse metabolic responses in the cell (1). The net result of insulin's interaction with its receptor is to alter the activity of rate-limiting enzymes in insulin-sensitive pathways by promoting post-translational modification of preexisting enzymes and alterations in the expression of specific gene products as well. Current concepts indicate that specific alterations in gene transcription result from a direct interaction of trans-acting factors with cis-acting sequences in the regulated gene (2). One approach to the problem of identifying mediators of the effect of insulin on gene expression is to identify an effect of the hormone on transcription of a specific gene, define cis-acting regulatory sequences in or near the gene that mediate the effect, and utilize these sequences to isolate and characterize presumed insulin-sensitive trans-acting factors.

The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase [GAPDH; D-glyceraldehyde-3-phosphate:NAD$^+$ oxidoreductase (phosphorylating), EC 1.2.1.12] is regulated by insulin in the cultured 3T3-F442A adipocyte cell line (3). GAPDH mRNA levels increase 10-fold in 3T3 adipocytes exposed to insulin (4). We have evidence that the inductive effect of insulin on GAPDH mRNA content in the 3T3 adipocyte reflects an increase in GAPDH gene transcription (M.C.A. and H. M. Goodman, unpublished results). In this study, we show that GAPDH mRNA content is also induced 3-fold when H35 hepatoma cells are exposed to physiologic concentrations of insulin. Thus, the GAPDH gene is regu-

lated in at least two cell lines derived from insulin-sensitive tissues, liver and fat.

There are 20–150 pseudogenes for GAPDH in the human genome and more than 200–300 in the rat genome (5–8). Previous attempts to isolate the GAPDH gene have resulted in isolation of processed pseudogenes (5–7). We have identified and cloned a human GAPDH gene with putative promoter sequences, introns, and translational control signals appropriate for a functional gene. In transiently transfected murine L-cell fibroblasts, the gene encodes an active GAPDH protein of appropriate molecular mass. When the intact gene is stably transfected into insulin-sensitive rodent cell lines, a correctly initiated human GAPDH mRNA is expressed, which can be regulated by insulin to the same extent as endogenous GAPDH mRNA (i.e., 10-fold in 3T3 adipocytes and 3-fold in H35 hepatoma cell lines). A construct containing the 5′-flanking sequences and promoter of the isolated human GAPDH gene fused to the reporter gene chloramphenicol acetyltransferase (CAT) is regulated by insulin in transfected 3T3 adipocytes and H35 hepatoma cell lines. These studies indicate that insulin regulates human GAPDH gene expression through a cis-acting element in the 5′-flanking sequences of the gene.

MATERIALS AND METHODS

Cell Culture Conditions. H35 hepatoma cells, obtained from John Koontz (University of Tennessee), were grown to confluence in Dulbecco–Vogt-modified minimal essential medium supplemented with 10% fetal bovine serum and 2 mM glutamine, incubated in serum-free medium supplemented with 0.1% crytalline bovine serum albumin and 10 mM Hepes (pH 7.4) for 24–48 hr, and then stimulated with insulin at 1 milliunit/ml for up to 24 hr. Serum starvation for up to 96 hr in the presence or absence of insulin had no significant effect on H35 hepatoma cell viability.

Confluent 3T3-F442A cells were differentiated in the presence of 10% fetal bovine serum, 0.1 mM isobutylmethylxanthine, 0.25 μM dexamethasone, and insulin at 10 μg/ml as previously described (4). Within 48 hr after removal of insulin from the media of fully differentiated monolayers (4), GAPDH mRNA levels declined to basal levels.

Plasmids. One million recombinant phage from a human EMBL3 genomic library (9) were screened with a full-length human GAPDH cDNA probe. One candidate, with 1.1 kilobases of 5′-flanking sequence, introns, and the complete human GAPDH coding sequence, was subcloned into pUC12 to yield pghGAPDH, which was used for transfection of L cells, 3T3-F442A preadipocytes, and H35 hepatoma cells. A 507-base-pair *Taq* I fragment, which contained bases −487 to

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus.
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+20 of the human GAPDH gene, was subcloned into pUC-CAT, a promoterless plasmid containing the CAT gene coding sequence (10); the fusion gene is referred to as HGAPDH–CAT. The neomycin-resistance plasmid (pSV2-neo) (11), Rous sarcoma virus (RSV)–CAT construct (12), and PXGH5, a plasmid in which human growth hormone gene expression is driven by the metallothionein I promoter (13), have been previously characterized.

Cellular Transfection Assays. pghGAPDH was transiently transfected and expressed in L cells by a modification of the DEAE-dextran protocol (14), as described by Selden *et al.* (13). Total RNA and cytosolic proteins were extracted, and the expression of human GAPDH mRNA, protein content, and enzymatic activity were quantitated as previously described (4).

H35 hepatoma cells and 3T3 preadipocytes were transfected by calcium phosphate-mediated DNA transfer as previously described (11). CAT activity driven by transfected fusion genes was assayed as previously described (12).

RNA Isolation and Analysis. Ten micrograms of total RNA, isolated from control and insulin-stimulated cells, was subjected to agarose/formaldehyde gel electrophoresis and RNA gel blot analysis as previously described (4).

The start site of transcription of mouse and human GAPDH mRNA was distinguished by first-strand synthesis (15) primed with a ^{32}P-labeled oligonucleotide complementary to the coding region of both mouse and human GAPDH mRNA (5′ CCATGTAGTTGAGGTCAATG 3′). Ten micrograms of total RNA was annealed to 2×10^6 cpm of probe in the presence of 0.1 mM $MgCl_2$, 2.5 mM dithiothreitol, 0.5 mM dNTP, 140 mM KCl, and 20 units of reverse transcriptase for 90 min at 43°C. The extension products were extracted with phenol, precipitated in the presence of 0.3 M sodium acetate/66% ethanol, resuspended in sequencing dye, matched for recovered counts, and subjected to electrophoresis on 8% urea/polyacrylamide gels (15). The dried gels were subjected to autoradiography overnight. This assay is referred to as a primer extension assay in the text.

The start site of transcription for the HGAPDH–CAT fusion mRNA was defined by S1 nuclease analysis (16).

RESULTS

To determine if the effect of insulin on GAPDH gene expression was mediated through cis-acting sequences in the gene, we isolated a functional insulin-regulatable GAPDH gene. One million recombinant phage from a human genomic DNA library were screened with a full-length human GAPDH cDNA probe; 63 positive clones were detected. Three independently isolated recombinant phage had a similar restriction map, which was consistent with the presence of a GAPDH gene that contained introns. Hybridization with a series of synthetic oligonucleotides spanning the length of the published human GAPDH cDNA sequence showed that one of these three genomic clones contained the intact coding sequence and 1.1 kilobases of 5′-flanking sequence in a 12-kilobase *Bam*HI restriction fragment.

Two independent lines of evidence, obtained from sequence analysis and expression of the cloned isolated DNA fragment, indicated that it encoded a functional GAPDH gene. First, sequence analysis showed that the gene contained the complete coding sequence and eight introns (L. Ercolani, B. Florence, M. Denaro, and M.C.A., unpublished results). By utilizing an intron as probe, only one human GAPDH gene was detected on a Southern blot. The coding sequence was in agreement with published human GAPDH cDNA sequences.

Second, results of transfection experiments showed that the fragment encoded a potentially functional GAPDH gene. The DNA fragment was transiently transfected into L cells,

and the expressed products were analyzed to determine if (*i*) transcription of human GAPDH mRNA was correctly initiated from the GAPDH promoter, (*ii*) human GAPDH mRNA and protein were appropriate in size, and (*iii*) the protein expressed was active. The start sites of transcription for endogenous human GAPDH mRNA expressed in human placenta and that of our purified transfected human GAPDH gene were identical as determined by a primer extension assay (Fig. 1, lanes A and C). Transfection with the 12-kilobase *Bam*HI fragment, therefore, resulted in transcription of an appropriately initiated human GAPDH mRNA. Human GAPDH mRNA expressed after transfection of L cells comigrated on agarose/formaldehyde gels with the endogenous human GAPDH mRNA expressed in human placenta (Fig. 1, compare lanes G–J with lane E). GAPDH mRNA expressed after stable transfection of H35 hepatoma cells directed the synthesis of human GAPDH protein, which comigrated on sodium dodecyl sulfate/polyacrylamide gels with endogenous human hepatoma GAPDH protein (Fig. 1, lanes L and M). Thus, both the mRNA and protein were appropriate in size. Finally, the 80% increase in total GAPDH mRNA expressed in transfected L cells (Fig. 1, lanes A and B) was accompanied by an 80% increase in GAPDH activity from 0.36 unit/mg of protein in untransfected L cells to 0.62 unit/mg in transfected L cells.

To determine if this functional human GAPDH gene could be regulated by insulin, it was stably transfected and expressed in the H35 hepatoma and 3T3-F442A preadipocyte cell lines. Three G418-resistant H35 hepatoma subclones, which expressed the human GAPDH gene, were exposed to insulin at 1 milliunit/ml for 16 hr, and total RNA was isolated. Under these conditions, rat GAPDH mRNA was induced 2- to 3-fold in untransfected H35 hepatoma cell lines (Fig. 2, lanes A and B), whereas actin mRNA was unaffected (Fig. 2 *Inset*). The effect of insulin on GAPDH mRNA could be demonstrated within 4 hr of exposure of cells to nanomolar concentrations of insulin (data not shown). Human GAPDH mRNA expressed in H35 hepatoma cells transfected with pghGAPDH was quantitated by RNA blot hybridization analysis by using a probe that distinguished the 5′-untranslated region of rodent and human GAPDH mRNAs (Fig. 1, lanes D and E). The product of the transfected human GAPDH gene (Fig. 2, lanes C–H) was induced 2- to 3-fold, which is similar to the results obtained with the endogenous rat gene (Fig. 2, lanes A and B).

In 3T3 adipocytes transfected with pghGAPDH (Fig. 3), the cellular content of mouse and human GAPDH mRNA was quantitated simultaneously by the primer extension assay. Both the endogenous mouse GAPDH (Fig. 3, lanes A–F) and the exogenous human GAPDH (Fig. 3, lanes C–F) mRNAs were induced 10-fold by insulin. This experiment demonstrated that the start site of transcription was the same for the control and insulin-induced species expressed by both the endogenous and transfected GAPDH genes. Taken together, these observations indicate that any cis-acting sequences required to mediate the effect of insulin on human GAPDH gene expression in cultured H35 hepatoma and 3T3 adipocytes were present in our genomic clone.

To localize sequences necessary for the insulin effect, the 5′-flanking region of the human GAPDH gene was fused to the CAT gene, and this construct was cotransfected with pSV2neo into H35 hepatoma cells. Two stable lines were isolated; one showed a 5-fold induction of CAT activity (Fig. 4A) when exposed to insulin. The other line showed a similar 6-fold inductive effect when exposed to insulin at 1 milliunit/ml and a half-maximal effect at 45 microunits/ml (data not shown). The time course and dose–response curve of the insulin effect was identical to that for endogenous GAPDH mRNA expressed in H35 hepatoma lines (data not shown) and 3T3 adipocyte lines (4). These data suggest that the effect of insulin
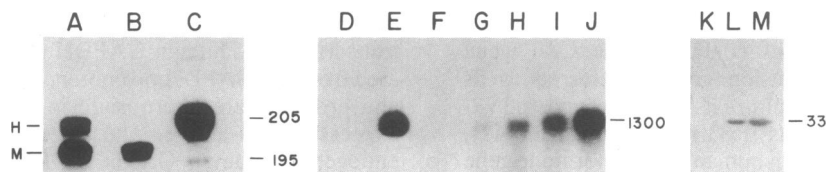
FIG. 1.   Characterization of the products expressed by the transfected human GAPDH gene. (*Left*) Four micrograms of pghGAPDH DNA was transiently expressed in mouse L cells by using the DEAE-dextran procedure (13). Total cytoplasmic RNA was isolated 48 hr later from cells transfected with the human GAPDH gene or from untransfected L cells. The content of human GAPDH mRNA in 10 μg of total RNA was quantitated by primer extension. The predicted sizes of the extended product for human (H) GAPDH mRNA and murine (M) GAPDH mRNA are 205 and 195 nucleotides (nt), respectively. Mouse L cells transfected with the human GAPDH gene (lane A) yield extension products of 205 and 195 nt, whereas untransfected cells (lane B) contain one product of 195 nt. Human placental RNA (lane C) contains one major extension product of 205 nt, which comigrates with that of the transfected human GAPDH gene. Thus, the start site of transcription is identical for both the transfected human and endogenous human GAPDH genes. (*Center*) Total cytoplasmic RNA was isolated from mouse L cells 48 hr after transfection with 0 (lane F), 2 (lane G), 4 (lane H), 8 (lane I), or 16 (lane J) μg of pghGAPDH as described above. Ten micrograms of total RNA was subjected to electrophoresis on an agarose/formaldehyde gel. Rodent GAPDH mRNA isolated from rat liver (lane D) and human GAPDH mRNA isolated from human placenta (lane E) were distinguished by use of an end-labeled synthetic oligonucleotide probe complementary to the 5′-untranslated region of human GAPDH mRNA nucleotides 4–64 (6), which does not detect rodent GAPDH mRNA (compare lanes D and E). Human GAPDH mRNA encoded by the human GAPDH transfected gene (lanes G–J) comigrated with human GAPDH mRNA (1300 nt) encoded by the authentic GAPDH gene expressed in human placenta (lane E). (*Right*) Cytoplasmic protein (50 μg) isolated from rat H35 hepatoma cells (lane K), a rat H35 hepatoma line stably transfected with the human GAPDH gene (lane L), or the human hepatoma G2 line (lane M) was subjected to electrophoresis on 10% sodium dodecyl sulfate/polyacrylamide gels and was transferred to nitrocellulose as previously described (17). The nitrocellulose filter was coated with 3% gelatin in TBS [150 mM NaCl/50 mM Tris·HCl (pH 7.5)] for 2 hr. A specific antibody raised to a unique human GAPDH peptide (human, His-Gln-Val-Val-Ser-Ser-Asp-Phe-Asn-Ser-Asp-Thr; rodent, Asp-Gln-Ala-Ala-Ser-Cys-Asp-Phe-Asn-Ser-Asn-Ser) was diluted 1:250 in 0.25% gelatin/TBS and was incubated with the filter for 3 hr at room temperature. The filter was washed with 0.1% Nonidet P-40/TBS, blocked again, and incubated with [^{125}I]-labeled *Staphylococcus aureus* (4 × 10^5 cpm/ml) for 2 hr at room temperature. The blot was washed in 0.5% Triton X-100/0.1% sodium dodecyl sulfate/TBS for 2 hr at room temperature, dried, and subjected to autoradiography overnight. The antibody does not detect GAPDH-related peptide sequences in rodent cells (lane K) but does detect a single GAPDH-related species in a rat H35 hepatoma stable line transfected with the human GAPDH gene (lane L), as described in Fig. 2. The protein product (33 kDa) encoded by the transfected gene (lane L) comigrates with human GAPDH protein expressed by the endogenous gene in the human hepatoma G2 line (lane M).

on the HGAPDH–CAT fusion gene and the endogenous GAPDH gene are mediated through identical pathways.

When the HGAPDH–CAT construct was stably transfected into 3T3 preadipocyte lines, CAT enzyme activity was induced 2- to 3-fold by insulin (Fig. 4B). Expression of an RSV–CAT fusion gene was slightly inhibited (30 to 50%) by insulin in the 3T3 adipocyte and was unaffected in H35 hepatoma cell lines (Fig. 4 *Insets*). This finding indicates that insulin did not affect CAT mRNA or enzyme stability through a posttranscriptional mechanism in these cell lines. The primer extension in Fig. 3, lanes A and B, showed that endogenous GAPDH mRNA was induced 10-fold in 3T3 adipocytes stably transfected with RSV–CAT, indicating that

the cells were indeed responsive to insulin and that the transcriptional machinery was not saturated by the presence of the RSV–CAT plasmid. Taken together, these findings strongly support the conclusion that the inductive effect of insulin on the HGAPDH–CAT fusion gene is specifically mediated through sequences in the 5′-flanking region of the human GAPDH gene.

The start site of transcription from the HGAPDH–CAT
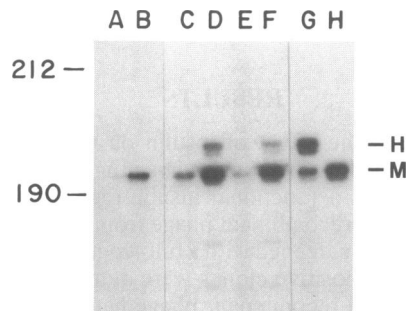
FIG. 3.   Insulin regulation of the intact human GAPDH gene in transfected mouse 3T3 adipocytes. pghGAPDH (10 μg) was cotransfected with PSV2neo (1 μg) into 3T3 preadipocytes as described in Fig. 2. Two 3T3 preadipocyte stable subclones selected with G418 at 300 μg/ml were grown to confluence, differentiated, and exposed to insulin at 1 milliunit/ml in the presence of 1% fetal bovine serum for 16 hr. Total RNA was isolated from control and insulin-stimulated pairs, and GAPDH mRNA content was quantitated by the primer extension assay in which human (H) GAPDH mRNA yields an extension product of 205 nt and murine (M) GAPDH mRNA yields an extension product of 195 nt. The products of the first-strand synthesis were separated on an 8% urea/polyacrylamide gel. The autoradiograph shows extension products of GAPDH mRNA isolated from control (lane A) and insulin-treated (lane B) 3T3 adipocytes transfected with the RSV–CAT construct used in Fig. 4B to show the extension products of the control and insulin-stimulated endogenous gene; control 3T3 adipocytes from two independent G418-resistant subclones transfected with pghGAPDH 7a (lane C) and 8a (lane E); insulin-treated 3T3 adipocytes from subclones 7a (lane D) and 8a (lane F); mouse L cells transfected with pghGAPDH (lane G); and untransfected mouse L cells (lane H) (from Fig. 1, lanes A and B).
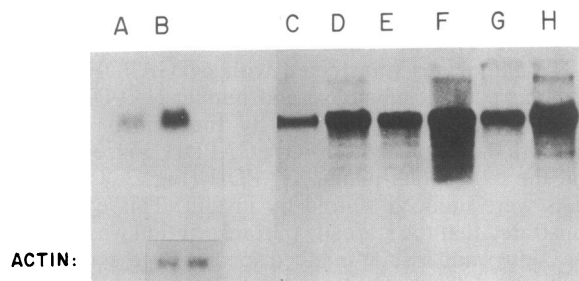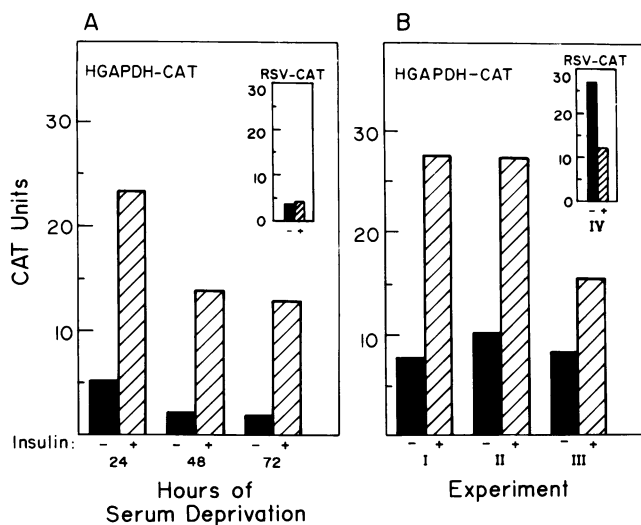

FIG. 2.   Insulin regulation of the endogenous rat GAPDH gene and transfected human GAPDH gene in the H35 hepatoma cell line. Rat GAPDH mRNA in untransfected control (lane A) and insulin-stimulated H35 hepatoma (lane B) cells was detected with a full-length human GAPDH cDNA probe. (*Inset*) Lanes A and B were rehybridized with an actin probe to show specificity of the insulin effect on GAPDH mRNA. H35 hepatoma cells (500,000 cells per plate) were cotransfected with pghGAPDH (10 μg) and pSV2neo (1 μg) by using the CaPO₄ precipitation protocol. Three independent stable subclones selected with G418 at 300 μg/ml (lanes C–H) were grown to confluence, serum-starved for 48 hr, and exposed to insulin at 1 milliunit/ml for 16 hr. Total cytoplasmic RNA (10 μg) prepared from control (lanes C, E, and G) or insulin-treated (lanes D, F, and H) pairs was subjected to blot-hybridization analysis to quantitate expression of exogenous human GAPDH-regulated sequences by using a probe specific for the 5′-untranslated region of human GAPDH mRNA (see Fig. 1, lanes D and E). The autoradiograph is shown.

FIG. 4. Regulation of the HGAPDH–CAT fusion gene in insulin-sensitive stable lines. (*A*) HGAPDH–CAT (10 μg) or RSV–CAT (10 μg) was cotransfected with pSV2neo (1 μg) into H35 hepatoma cells, and stable lines were selected with G418. In this experiment, one of two subclones expressing HGAPDH–CAT activity was grown to confluence and was serum-starved for a total of 24 hr, 48 hr, or 72 hr. (*Inset*) A pooled stable line transfected with RSV–CAT was serum-starved for a total of 72 hr. Insulin was added at a final concentration of 1 milliunit/ml for the last 16 hr of the incubation. The effect of insulin on the expression of specific genes in hepatoma cells is accentuated by prior serum starvation. In this experiment, basal CAT activity progressively declined over the course of 72 hr of serum deprivation; however, the inductive effect of insulin on HGAPDH–CAT expression was evident after only 24 hr of serum deprivation. CAT activity was expressed in units (1 unit = percentage of [^{14}C]chloramphenicol acetylated per 4 hr per 100 μg of extract protein). (*B*) HGAPDH–CAT (10 μg) or RSV–CAT (10 μg) was cotransfected with PSV2neo (1 μg) into 3T3-F442A adipocytes and stable lines selected with G418. Pooled stable lines expressing the HGAPDH–CAT fusion gene or the RSV–CAT fusion gene (*Inset*) were grown to confluence and differentiated. The cells were incubated in 1% fetal bovine serum with or without insulin at 1 milliunit/ml for 16 hr. CAT activity was measured and expressed in units (1 unit = percentage of [^{14}C]chloramphenicol acetylated per 4 hr per milligram of extract protein). The effect of insulin on the expression of GAPDH mRNA in 3T3 adipocytes is seen with or without serum deprivation (4).

fusion gene was mapped by S1 nuclease analysis of stably transfected cells (Fig. 5). The predicted size of the S1 nuclease-protected fragment initiated from the human GAPDH promoter was 116 nucleotides. We detected an insulin-induced band that comigrated with a marker 110 nucleotides in length in both H35 hepatoma and 3T3 adipocyte stable lines.

HGAPDH–CAT was transiently transfected and expressed in H35 hepatoma cells (Table 1). PXGH5, a reporter gene that encodes human growth hormone, was included in early experiments to correct for anticipated plate-to-plate variations in transfection efficiency. Insulin induced CAT activity 2.5- to 4-fold when expression was driven by the human GAPDH promoter. The expression of PXGH5 and RSV–CAT was slightly inhibited by insulin. Thus, the specific inductive effect of insulin on the HGAPDH–CAT fusion gene could be demonstrated in stable and transient transfection systems, indicating that the effect was independent of the chromosomal location of the transfected gene.

DISCUSSION

This study maps the inductive effect of insulin on the glycolytic enzyme GAPDH to sequences in the 5'-flanking
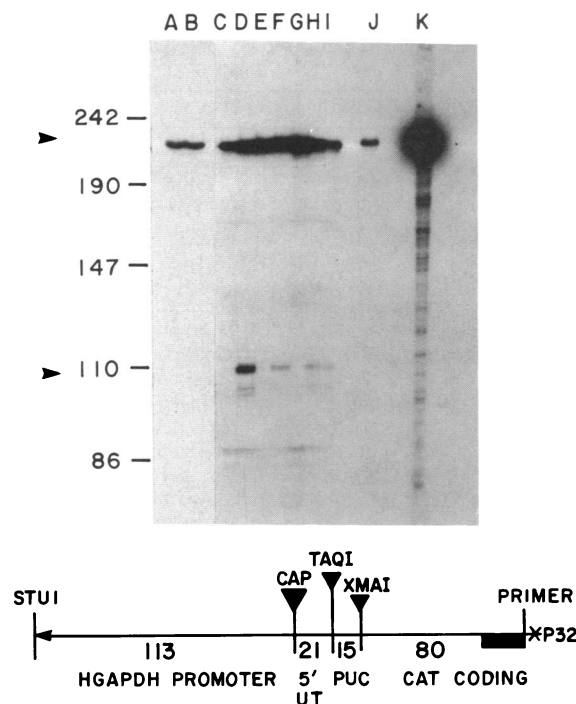

FIG. 5. (*Upper*) Detection of correctly initiated HGAPDH–CAT mRNA transcripts by S1 nuclease protection assay. A 20-base oligonucleotide complementary to the coding sequence of CAT was used to prime the synthesis of an end-labeled, single-stranded DNA probe, which included 95 bases of CAT coding and vector-related sequences and 133 bases of human GAPDH-related sequences. Total RNA (40 μg) was isolated from the control and insulin-treated H35 hepatoma or 3T3-F442A adipocyte stable lines transfected with HGAPDH–CAT (see Fig. 4) and was hybridized to 0.15 pmol of probe (16) after which the RNA–DNA hybrids were digested with 6 units of S1 nuclease (16), extracted with phenol, precipitated, and subjected to electrophoresis on an 8% urea/polyacrylamide gel (15). The dried gel was subjected to radioautography for 10 days. Lanes: A and B, control and insulin-treated samples, respectively, from untransfected H35 hepatoma cells; C and D, control and insulin-treated pairs, respectively, from two independent H35 hepatoma stable lines; E and F, control and insulin-treated pairs, respectively, from two independent H35 hepatoma stable lines; G, H, and I, transfected 3T3 adipocytes exposed to insulin (1 milliunit/ml) for 0, 4, and 8 hr, respectively; J, no cellular RNA; K, no cellular RNA and no S1 nuclease digestion. (*Lower*) Restriction map of the human GAPDH promoter–CAT coding region. UT, untranslated; PUC, polylinker sequences.

region of the gene. The effect of insulin on expression of the chimeric gene HGAPDH–CAT was compared to that of insulin on a chimeric RSV–CAT construct in transiently and stably transfected insulin-sensitive cell lines. Cells exposed to physiologic concentrations of insulin showed a 2- to 3-fold increase in expression of the HGAPDH–CAT fusion gene product in 3T3 adipocytes and a 5- to 8-fold increase in HGAPDH–CAT activity in the H35 hepatoma cell line, whereas expression of the RSV–CAT fusion gene product was unchanged or inhibited by insulin. These studies clearly show that specific cis-acting sequences in the 5'-flanking region of the human GAPDH gene mediate the inductive effect of insulin on GAPDH gene expression independent of its position in chromatin.

We have observed a rapid increase in GAPDH gene transcription in 3T3 adipocytes exposed to insulin. GAPDH gene transcription increases within 30 min of insulin exposure and reaches a maximum of 10-fold within 60 min (M.C.A. and H. M. Goodman, unpublished results). This study shows that GAPDH mRNA levels are regulated by insulin in a second insulin-sensitive tissue, the H35 hepatoma cell line. Although we have not been able to accurately measure the transcription of this gene in hepatoma cells or liver (because the rates

Table 1. Effect of insulin on the transient expression of CAT directed by the human GAPDH promoter and RSV promoter in H35 hepatoma cells

Plasmid(s)	Activity		Induction, % control
	Control	Insulin	
HGAPDH–CAT	5.7*	15.0*	260
	0.9*	3.9*	400
HGAPDH–CAT	0.7*	2.3*	330
and PXGH5	1.5†	1.3†	87
RSV–CAT	3.8*	1.9*	50

H35 hepatoma cells were trypsinized 24 hr prior to transfection, plated at a density of 5×10^5 cells per 60-mm plate, and transiently transfected with calcium phosphate precipitates containing HGAPDH–CAT (20 µg), RSV–CAT (20 µg), or HGAPDH–CAT (12.5 µg) in combination with PXGH5 (12.5 µg) for 4 hr as described above. The cells were shocked with 20% (vol/vol) dimethyl sulfoxide in phosphate-buffered saline for 2 min. Confluent monolayers were serum-starved for 24–48 hr. When PXGH5 was used to correct for DNA uptake, samples of medium were taken from each plate and assayed for human growth hormone immunoreactivity prior to the addition of hormones. Triplicate plates were exposed to no hormone or insulin at 1 milliunit/ml for 16 hr, and samples taken for analysis of CAT and growth hormone gene expression. Human growth hormone in the medium was assayed by using a Nichols radioimmunoassay kit, which is linear within a range of 0.5–50 ng of human growth hormone per ml.
*CAT activity in units (1 unit = percentage of [^{14}C]chloramphenicol acetylated in 4 hr per 100 µg of protein).
†Human growth hormone activity (in ng/ml).

are less than 8 ppm of newly synthesized RNA), this study strongly suggests that insulin alters the transcription of this gene in both 3T3 adipocytes and H35 hepatoma cells.

Other groups have shown that insulin can alter the transcription of specific genes. The effects of insulin on gene transcription can be categorized as those that counteract the effect of catabolic hormones (and cAMP) and those that promote energy storage and cell growth. The best-studied model of the first effect is insulin inhibition of cAMP-stimulated phospho*enol*pyruvate carboxykinase gene expression in hepatoma cell lines (18, 19). Insulin inhibits cAMP-stimulated gene transcription and decreases basal transcription by 50–75% as well (19). In contrast, both cAMP (20) and insulin (21) activate c-*fos*, c-*myc*, and actin gene transcription. The content of pancreatic amylase mRNA is dramatically decreased in diabetic animals and returns to control levels when insulin is replaced (22). Although a change in gene transcription has not been directly demonstrated, preliminary evidence indicates that 5′-flanking sequences in the amylase gene mediate the effect (M. Meisler, personal communication). Weak inhibition of growth hormone gene transcription by insulin and insulin-like growth factors has been observed in GH3 cells (23).

Our goal is to understand how insulin activates the transcription of specific genes. Peptide hormones that rapidly modulate enzymatic activity by altering the phosphorylation state of specific gene products may achieve a more prolonged effect on metabolic processes by modulating the phosphorylation state of trans-acting factors that regulate the transcription of the same genes (24). It is tempting to speculate that the ability of insulin to stimulate phospho–dephospho interconversions will mediate some of its effects on gene expression. For example, insulin may activate a trans-acting factor that regulates GAPDH gene transcription by stimulating protein phosphorylation, whereas insulin inhibition of phospho*enol*pyruvate carboxykinase gene transcription could be explained by insulin's well-described ability to promote net dephosphorylation of cAMP-regulated phosphoproteins (25). We expect that isolation and characterization of

the putative insulin-regulated trans-acting factor that interacts with the GAPDH gene will provide a useful tool for further studies aimed at elucidating the steps intermediate to activation of the insulin receptor tyrosine kinase and insulin-induced alterations in specific gene expression.

Note. While this manuscript was under review, we received a preprint describing multihormonal regulation of a chimeric gene containing sequences −660 to +69 of the phospho*enol*pyruvate carboxykinase gene fused to CAT; this construct is induced 2-fold by cAMP, and this effect is inhibited by insulin (26).

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