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Transcriptional and Posttranscriptional Regulation of Tyrosine Aminotransferase by Insulin in Rat Hepatoma Cells†

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ABSTRACT: The molecular mechanisms of induction of tyrosine aminotransferase (TAT) by insulin were studied in the well-differentiated rat hepatoma cell line Fao. Incubation of Fao cells with insulin resulted in a 2-fold increase in TAT activity and TAT mRNA measured by Northern blot analysis with an oligonucleotide probe to the 5′ end of the gene. The effect of insulin on TAT activity had a lag period of 30–60 min and was maximal within 4–5 h. The insulin effect on TAT mRNA was rapid, half-maximal after 15 min, and complete within 1–2 h. Insulin dose–response curves for stimulation of TAT activity and TAT mRNA were almost identical. TAT mRNA levels and enzyme activity were also stimulated by anti-insulin receptor antibodies and dexamethasone but not by wheat germ agglutinin, concanavalin A, or phytohemagglutin. The effect of insulin on the TAT gene was further investigated by measuring the relative rate of transcription in isolated nuclei using genomic TAT clones. Insulin produced a 1.5–1.7-fold increase in the production of TAT RNA transcripts. Dexamethasone induced both TAT activity and TAT mRNA to a comparable extent. In the presence of dexamethasone, insulin produced an additional 2-fold stimulation of TAT activity but had no additional effect on the abundance of TAT mRNA. These data provide direct evidence that insulin can increase TAT activity by at least two distinct mechanisms: insulin alone appears to increase TAT activity and TAT mRNA due to a stimulation of the TAT gene transcription rate; while in the presence of glucocorticoids, insulin increases TAT activity but not TAT mRNA, suggesting an insulin effect at the posttranscriptional level.

Tyrosine aminotransferase (TAT,[1] L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) is a liver-specific gluconeogenic enzyme. The activity of tyrosine aminotransferase is regulated by various hormones including insulin, glucagon, and glucocorticoids [for a review, see Granner and Hargrove (1983)]. The mechanisms of insulin action in the regulation of TAT

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[1] Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; 1× SSC, 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0; TAT, L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5); DEX, dexamethasone; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; WGA, wheat germ agglutinin; kb, kilobase(s).

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activity are complex and still not clear. Insulin increases TAT activity in rat liver (Holten & Kenney, 1967; Hager & Kenney, 1968) and in various cell lines derived from rat hepatoma Reuber H35 (Reel et al., 1970; Lee et al., 1970; Barnett & Wicks, 1971; Iwamoto et al., 1981; Crettaz & Kahn, 1983) or Morris 7288C (Gelehrter & Tomkins, 1970; Gelehrter et al., 1972; Heaton & Gelehrter, 1980; Iwamoto et al., 1981; Crettaz & Kahn, 1983). In rat hepatoma H35 cells, insulin increases TAT activity by increasing the rate of enzyme synthesis (Reel et al., 1974), whereas in rat HTC hepatoma cells exposed to glucocorticoids, insulin increases TAT activity by selectively retarding the degradation rate of the enzyme (Spencer et al., 1978). In the liver of adrenalectomized rats, it has been shown that insulin increases TAT activity by increasing the concentration of TAT mRNA (Hill et al., 1981) and the transcription rate of the TAT gene (Lee et al., 1986). However, in cultured cells, it has not been shown directly if insulin increases TAT activity by increasing the abundance of TAT mRNA and if these effects are altered by dexamethasone.

We have studied the well-differentiated rat hepatoma cell line Fao to determine whether insulin increases TAT activity by increasing the TAT mRNA concentration and synthesis and to determine if glucocorticoids modify the insulin effect. We demonstrate directly that insulin stimulates TAT activity by at least two distinct mechanisms. Insulin alone increases TAT activity by increasing the amount of TAT mRNA due to increases in transcription rate. In the presence of glucocorticoids, however, insulin increases TAT activity but has no additional effect on the level of TAT mRNA, suggesting a site of insulin action posttranscriptionally.

Experimental Procedures

Materials. Cell culture medium RPMI 1640 and fetal bovine serum were from GIBCO; all other tissue culture supplies were from Nunc or Costar. Porcine insulin was from Elanco, guanidine thiocyanate from Fluka, AG (Buchs, OH), agarose from Bethesda Research Laboratories, cesium chloride from Varlacoid, Inc., formamide from EM Science, RNase-free DNase I from Worthington Biochemical Corp. (Freehold, NJ), proteinase from Boehringer Mannheim (Indianapolis, IN), [γ-^{32}P]ATP from New England Nuclear (Boston, MA), and [α-^{32}P]UTP from Amersham (Chicago, IL). All other chemicals were of analytical grade and were obtained from Sigma or Fisher Scientific. BA-85 nitrocellulose paper was purchased from Schleicher & Schuell. Fao is a well-differentiated subclone derived from Reuber H35 rat hepatoma (Reuber, 1961). The cells were grown in RPMI 1640 medium and supplemented with 5% fetal bovine serum.

TAT Assay. Cells were scraped in phosphate-buffered saline and collected by centrifugation. An aliquot was taken for the measurement of TAT activity by the method of Diamondstone (1966). One unit of TAT activity catalyzed the formation of 1 μmol of *p*-hydroxyphenylpyruvate/min at 37 °C. Proteins were determined according to the method of Bradford (1976).

Analysis of Cellular TAT mRNA Levels. Total RNA was prepared by the guanidinium–cesium chloride method of Chirgwin (1979). Ten micrograms of purified total RNA was separated by electrophoresis in a formaldehyde-containing 1% agarose gel and transferred to nitrocellulose paper as described by Thomas (1980). Alternatively, dot blots were prepared by spotting 5–20 μg of purified total RNA onto nitrocellulose filters, using a minifold apparatus (Schleicher & Schuell). In either case, the nitrocellulose paper was hybridized with 25 mL of solution containing (3–5) $\times$ 10^6 dpm/mL radiolabeled TAT-specific oligodeoxyribonucleotide probe (Maniatis et al., 1982). This probe consisted of 27 bases which had been synthesized complementary to the sequence of the 5'-end region of the rat TAT gene (Shinomiya et al., 1984) and labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase (Maniatis et al., 1982) to a specific activity of 4 μCi/pmol. The relative mRNA levels were quantitated by scanning densitometry of autoradiograms, basal mRNA levels being 1 arbitrary unit.

Transcription of TAT RNA in Isolated Nuclei. Cell nuclei were prepared by homogenization of the cells (10- or 15-cm dish) in 4 mL of 0.32 M sucrose, 3 mM $CaCl_2$, 2 mM magnesium acetate, 0.1 mM EDTA, 0.1% Triton X-100, 1 mM dithiothreitol, 0.1 trypsin inhibitor unit (TIU)/mL aprotinin, 0.1 mM phenylmethanesulfonyl fluoride, and 10 mM Tris-HCl, pH 8.0, using 10 strokes of the loose pestle of a Dounce homogenizer. The homogenate was diluted with 1 volume of 1.8 M sucrose, 5 mM magnesium acetate, 0.1 mM EDTA, 1 mM dithiothreitol, and 10 mM Tris-HCl, pH 8.0, and layered over 3 mL of 1.8 M sucrose solution. Nuclei were collected by centrifugation in an SW 41 rotor at 30000*g* for 45 min at 4 °C (Marzluff & Huang, 1984). Isolated nuclei were resuspended in reaction buffer. Either nuclei were counted or an aliquot was diluted in 1% SDS and the absorbance at 260 nm measured. Transcription was performed in 100 μL of reaction buffer containing (5–10) $\times$ 10^6 nuclei (2–4 absorbance units at 260 nm). The reaction buffer contained final concentrations of 16% glycerol, 60 mM HEPES, pH 7.0, 3 mM $MnCl_2$, 2 mM dithiothreitol, 3 mM magnesium acetate, 25 mM ammonium sulfate, 5 mM NaF, 9 μM creatine phosphate, 16 μg/mL creatine phosphokinase, 0.4 mM each of ATP, GTP, and CTP, and 200–250 μCi of [α-^{32}P]UTP (410 Ci mmol^{-1}) (Marzluff & Huang, 1984). Incubations were carried out at 26 °C for 45 min. The nuclei were then digested by the addition of 100 μg/mL RNase-free DNase I, 1 mM $CaCl_2$, and 100 μg of yeast tRNA for 15 min at 37 °C. Samples were then adjusted to 5 mM EDTA, 0.5% sodium dodecyl sulfate, and 50 μg/mL proteinase K and incubated at 25 °C for 45 min (Shupnik et al., 1985). Labeled RNA was purified by the guanidinium–cesium chloride method (Chirgwin et al., 1979) and precipitated in ethanol. The RNA transcripts were analyzed by using immobilized genomic TAT clones (Shinomiya et al., 1984). Plasmid DNAs were linearized with *Eco*RI restriction endonuclease, dissolved in 0.2 M NH_4OH and 2 M NaCl (final concentration 50 μg/mL), and heated to 100 °C for 2 min, and 40 μL (containing 0.6 μg each of pTAT EH 0.95 and pTAT EE 1.05 clone and 0.8 μg of pTAT EH 2.45 clone or 2 μg of pUC8) was spotted onto nitrocellulose filters using a Minifold apparatus (Schleicher & Schuell). The filter was baked at 80 °C for 2 h, and the spots were cut out. Hybridizations were performed at 45 °C for 72 h, and the filters were washed and treated as described (McKnight & Palmiter, 1979). The filters were autoradiographed for various periods of time, and the intensity of spots was determined by scanning densitometry.

Results

Effect of Insulin on TAT Activity and mRNA Concentration in Rat Hepatoma Cells. As we have previously reported (Crettaz & Kahn, 1984), Fao hepatoma cells possess a large number of high-affinity receptors for insulin and respond to the hormone for a large variety of effects including stimulation of TAT activity. The time course of the insulin effect on TAT activity and TAT mRNA was investigated by using cells which had been incubated for 18 h in serum-free medium, and then 100 nM insulin was added for various periods of time. Insulin stimulated TAT activity maximally 2-fold (Figure 1, closed

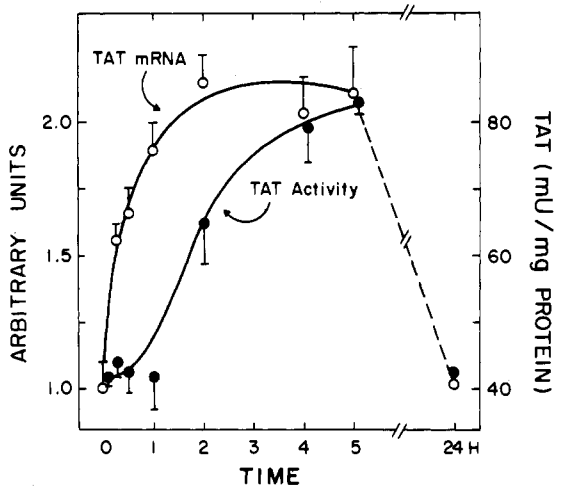

FIGURE 1: Time course of insulin effect on TAT activity and TAT mRNA in rat hepatoma cells. Fao cell monolayers were incubated for 18 h in serum-free medium. Insulin (100 nM) was added as a 100-fold concentrated solution for various periods of time. TAT activity (●) and TAT mRNA (○) were determined in cells from the same dish. TAT activity and TAT mRNA were analyzed as described under Experimental Procedures. The relative amount of TAT mRNA was determined by scanning densitometry of the autoradiograms. Each point is the mean of three determinations, ±SEM.

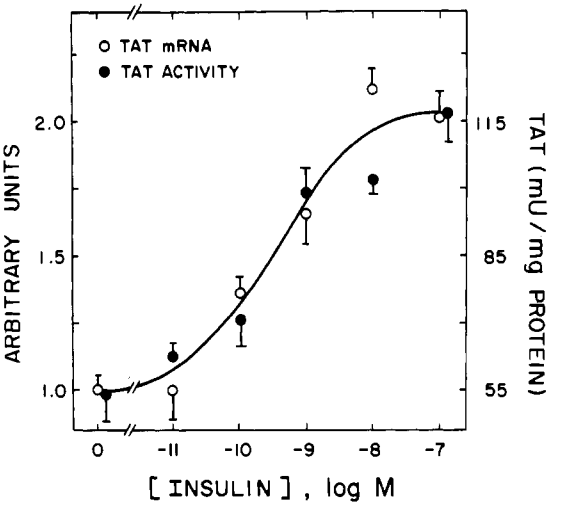

FIGURE 2: Dose–response curves of insulin effects on TAT activity and TAT mRNA in rat hepatoma cells. Fao cell monolayers were incubated for 18 h in serum-free medium. Insulin was added in various concentrations for 2.5 h. TAT activity (●) and TAT mRNA (○) were determined as described in Figure 1. Each point is the mean of four determinations, ±SEM.

circles). This insulin effect occurred after a lag period of 30–60 min and was maximal at 5 h.

To examine whether this effect of insulin on TAT activity in Fao cells is due to a concomitant increase in TAT mRNA concentration, the level of TAT mRNA was measured from the same dish. Total RNA was isolated and spotted directly on nitrocellulose filters as well as separated by electrophoresis on a formaldehyde-containing 1% agarose gel with subsequent transfer to nitrocellulose paper (Maniatis et al., 1982). The nitrocellulose filters were hybridized (Maniatis et al., 1982) with a radiolabeled synthetic oligonucleotide which is complementary to 27 bases of the 5′-end region of the rat TAT gene. On Northern blot analysis, TAT mRNA consisted of a single species with a length of approximately 2.4 kb. Incubation of Fao cells with 100 nM insulin alone resulted in a rapid 2-fold increase in the level of TAT mRNA as quantitated by scanning densitometry (Figure 1, open circles). This effect of the hormone was half-maximal within 15 min and maximal after 2 h. Thus, the effect of insulin on TAT mRNA preceded the increase of TAT activity by approximately 60 min, while the level of stimulation was the same magnitude.

We and others have shown that prolonged incubation of hepatoma cells with insulin results in a desensitization of hepatoma cells to various effects of insulin (Heaton & Gelehrter, 1980, 1981; Crettaz & Kahn, 1984). In this study, longer exposure of the Fao cells to insulin up to 24 h led to a parallel decrease in both TAT activity and mRNA (Figure 1). No change in the base-line level of TAT mRNA was observed at any time point.

To examine whether the TAT activity and mRNA levels respond to similar levels of insulin, the effects of various concentrations of insulin on the increase of TAT activity and TAT mRNA were studied (Figure 2). Both TAT activity (closed circles) and mRNA (open circles) were stimulated maximally at an insulin concentration of 100 nM. The half-maximal effect of insulin was at a concentration of approximately 0.3 nM. The insulin dose–response curves for TAT activity and TAT mRNA were virtually superimposable.

Effect of Anti-Insulin Receptor Antibodies and Lectins on TAT Activity and TAT mRNA in Rat Hepatoma Cells. Sera from patients with acanthosis nigricans and insulin resistance

Table I: Effect of Insulin, Anti-Insulin Receptor Antibodies (B-9), and Lectins on TAT mRNA and Activity in Fao Hepatoma Cells[a]

	TAT activity (x-fold stimulation)	TAT mRNA (x-fold stimulation)
basal	1.0	1.0
insulin (100 nM)	1.9–2.0	1.8–2.0
anti-receptor serum (dilution 1:100)	1.5–1.9	1.4–1.8
WGA (50 μg/mL)	1.0–1.1	0.8–1.2

[a] Cells were incubated in serum-free medium for 18 h. Insulin, anti-insulin receptor antibodies (B-9), and wheat germ agglutinin (WGA) were added for 4 h. TAT activity and TAT mRNA were measured as described in Figure 1. Duplicate determinations are indicated.

contain antibodies to the insulin receptor which inhibit insulin binding and mimic several effects of insulin (Kahn et al., 1977). To determine whether the insulin effect on TAT activity and its mRNA is mediated via binding and activation of the insulin receptor by the hormone, cells were incubated for 18 h in serum-free medium, and insulin receptor antibody containing B-9 serum was added in a 1:100 dilution for a further 4 h. Similar to insulin, the anti-receptor serum increased both the TAT activity and the TAT mRNA by 1.5–1.9 and 1.4–1.8 times, respectively (Table I). Therefore, it is likely that insulin exerts its effect on the activity and mRNA expression of TAT by binding and activation of the insulin receptor.

Various lectins have also been shown to have insulin-like effects and to stimulate TAT activity in the rat hepatoma H35 cells (Smith & Liu, 1981). When Fao cells were incubated with wheat germ agglutinin, concanavalin A, or phytohemagglutin for 4 h, however, there was no change in either the activity or the mRNA level of TAT. In Fao cells, lectins also fail to induce an insulin-like effect on glycogen synthase activity (unpublished observation).

Effect of Insulin on the Transcription Rate of the TAT Gene. In order to determine whether the increase of TAT mRNA produced by insulin is the consequence of a stabilization of TAT mRNA or an increase in the rate of TAT gene transcription, in vitro transcription by isolated nuclei was performed with [α-^{32}P]UTP as a radioactive RNA precursor. In this system, the synthesis of RNA is due to elongation and

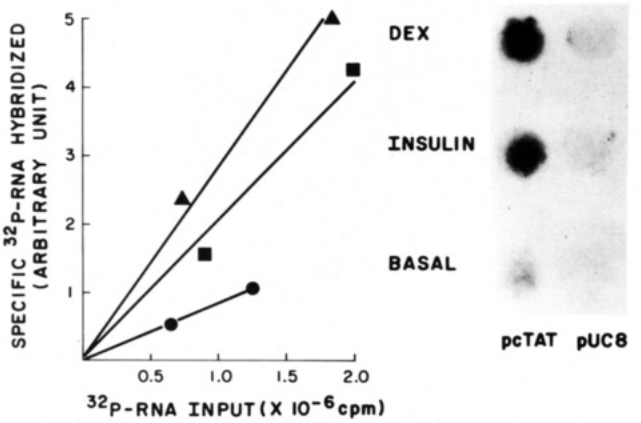

FIGURE 3: Effect of insulin and dexamethasone on the relative rate of transcription of the TAT gene. Cells were incubated in serum-free medium for 18 h. Insulin (100 nM) and dexamethasone (1 μM) were added for 2 h. Nuclei were isolated and allowed to continue RNA synthesis in transcription reactions containing [α-^{32}P]UTP, and [^{32}P]RNA transcripts were isolated. Two different input amounts of [^{32}P]RNA were hybridized to filters containing genomic TAT (pcTAT) subclones and pUC8. The filters were autoradiographed, and the activity was measured by scanning densitometry. On the left, specific hybridized [^{32}P]RNA was the difference between total (pcTAT) and nonspecific (pUC8) [^{32}P]RNA. Nonspecific was 6–17% of total. No addition (●); insulin (■); dexamethasone (DEX) (▲). On the right, an autoradiogram of the filters is shown. For further details, see Experimental Procedures.

completion of previously initiated RNAs with almost no reinitiation (Reeder & Roeder, 1972), and therefore, this should reflect the rate of RNA initiation which had occurred in the intact cell. The synthesis of TAT mRNA by isolated nuclei from control and insulin-treated cells was measured by hybridization of labeled transcripts to immobilized DNA of three clones containing sequences of the TAT gene (Hashimoto et al., 1984). The specificity of the hybridization was evaluated in several different ways. First, hybridization to filters containing immobilized plasmid pUC8 DNA was negligible. Second, hybridization of labeled transcripts was inhibited to over 90% by the addition of RNA prepared from differentiated hepatoma cells, while RNA from dedifferentiated hepatoma H5 cells with no TAT activity (Crettaz & Kahn, 1983) or human lymphocytes had no effect. Third, in each experiment, nuclear transcripts from glucocorticoid-treated cells were included as a positive control. The sensitivity of the hybridization assay was assessed by hybridizing various amounts of labeled transcripts to filters, and hybridization was proportional to the input labeled RNA (Figure 3). Nuclei isolated from cells treated for 2 h with insulin showed a 1.5–1.7-fold higher transcriptional rate of the TAT gene when compared to nuclei from control cells (Figure 3). Dexamethasone treatment for 2 h, used as a positive control, also increased the rate of transcription of the TAT gene by 2.2–2.5-fold. Thus, insulin appears to stimulate TAT activity by increasing the amount of TAT mRNA activity at the transcriptional level.

Effects of Insulin and Dexamethasone on TAT Activity and TAT mRNA in Rat Hepatoma Cells. It has been shown that insulin and dexamethasone have additive (Barnett & Wicks, 1971) or synergistic (Reel et al., 1970; Heaton & Gelehrter, 1980; Crettaz & Kahn, 1983) effects on TAT activity, suggesting that these two hormones act by different mechanisms. To determine if glucocorticoids modify the insulin action on tyrosine aminotransferase in Fao cells, we have studied the effect of insulin and dexamethasone on the concentration of TAT activity and TAT mRNA (Figure 4). After incubation of Fao cells in serum-free medium for 18 h, dexamethasone (1 μM) was added for 16 h in order to reach a maximal effect. Then insulin (100 nM) was added for another 4 h. Insulin and dexamethasone alone stimulated TAT activity by 2- and 10-fold, respectively, with a concomitant increase in TAT mRNA. When insulin was added to the dexamethasone-treated cells, both hormones had a synergistic effect on TAT activity, but insulin had no additional effect on the amount of TAT mRNA. Thus, in the presence of glucocorticoids, insulin stimulates TAT activity without a further increase in TAT mRNA.

Discussion

The mechanisms of insulin action in the regulation of TAT activity appear to be complex. Using the well-differentiated rat hepatoma cell line Fao, we find that insulin alone stimulates TAT activity by increasing the level of TAT mRNA. Insulin increases TAT mRNA and enzyme activity both to the same extent, and insulin dose–response curves for TAT activity and mRNA are almost superimposable. The insulin effects can

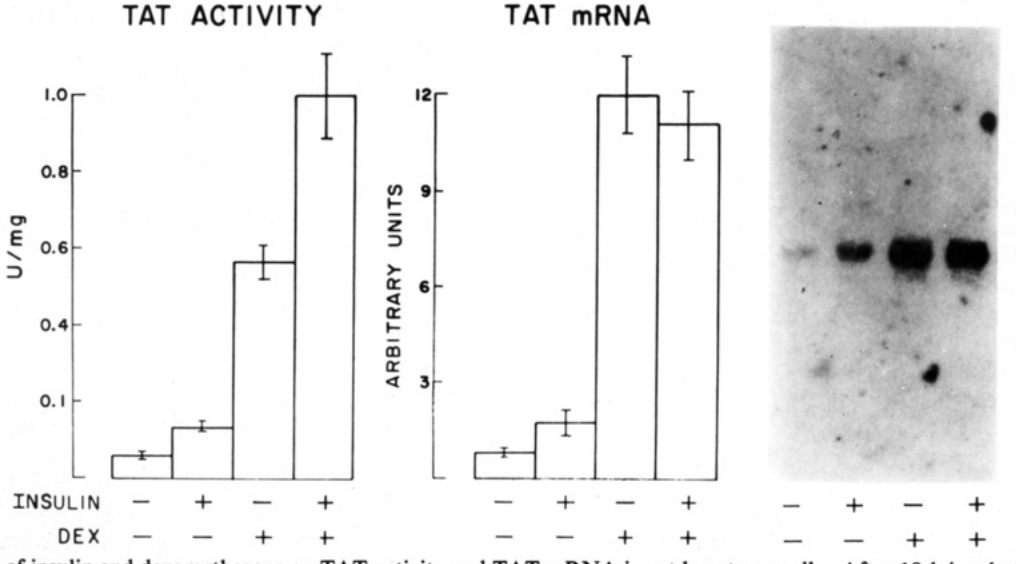

FIGURE 4: Effect of insulin and dexamethasone on TAT activity and TAT mRNA in rat hepatoma cells. After 18-h incubation of Fao cells in serum-free medium, dexamethasone (1 μM) was added for a further 16 h. Insulin (100 nM) was then added for 4 h. TAT activity and TAT mRNA were measured as described in Figure 1. Each bar represents the mean value of three determinations ± SD. An example of an autoradiogram of a nitrocellulose filter is shown on the right. 10 μg of total RNA was applied to each lane. The mRNA of TAT is approximately 2.4 kb in size.

be mimicked by anti-insulin receptor antibodies, indicating that insulin acts on TAT via binding and activation of the insulin receptor, causing an accumulation of TAT mRNA. In addition, we found that an increase in TAT mRNA is associated with an increase in the transcription rate of the aminotransferase gene, extending the observations of Lee et al. (1986) with liver from adrenalectomized rats to isolated cells. TAT is the first gene for which a positive effect of insulin on transcription has been shown. Inhibitory effects have been shown on the transcription rates of the phosphoenolpyruvate carboxykinase gene (Granner et al., 1983), the growth hormone gene (Yamasita & Melmed, 1986), and the albumin gene (Straus & Takemo, 1987). The insulin effect on the TAT mRNA expression is rapid and half-maximal after 15 min, whereas the induction of the enzyme activity has a lag period of 30–60 min. However, as total RNA was used for hybridization, the amount of TAT mRNA measured at early time points might reflect total TAT transcripts rather than mature mRNA. It is possible that this rapid increase in TAT mRNA is due to a stabilization of preexisting mRNA molecules. Further studies are needed to explore this possibility.

The mechanism of insulin action on TAT in the presence of glucocorticoids appears to be different. Insulin and dexamethasone alone stimulated TAT activity and mRNA in a proportional manner. When insulin was added to dexamethasone-treated cells, both hormones had a synergistic effect on TAT activity. A synergistic effect of insulin and dexamethasone on TAT activity has also been observed in rat hepatoma cell lines H35 (Reel et al., 1970) and HTC (Gelehrter & Tomkins, 1970; Gelehrter et al., 1972; Heaton & Gelehrter, 1982; Crettaz & Kahn, 1983). However, in the presence of glucocorticoids, insulin had no additional effect on the concentration of TAT mRNA. Thus, insulin stimulates TAT activity in glucocorticoid-treated cells without a concomitant alteration in the amount of TAT mRNA.

The mechanism of this posttranslational effect of insulin on TAT has not yet been clarified. In glucocorticoid-pretreated HTC cells, insulin specifically impairs the degradation rate of this enzyme, and this appears to account for the stimulatory effect of the hormone on TAT activity (Spencer et al., 1978). In contrast to well-differentiated rat hepatoma Fao cells, the HTC cells are only partially differentiated and have preserved only some normal liver functions (Crettaz & Kahn, 1983). Therefore, it is possible that the ability of insulin alone to induce the TAT gene has been lost in HTC cells. The posttranslational effect of insulin on TAT might also be operative when insulin is acting alone, since the stimulatory effects of insulin on TAT activity cannot be blocked completely by the RNA synthesis inhibitor actinomycin D in the well-differentiated rat hepatoma cell line H35 (Lee et al., 1979).

In summary, this study provides direct evidence that insulin may stimulate tyrosine aminotransferase by at least two distinct mechanisms. Insulin alone increases TAT activity and TAT mRNA due to a stimulation of the TAT gene transcription. In the presence of glucocorticoids, however, insulin increases TAT activity but does not further increase TAT mRNA, suggesting an insulin effect at the posttranscriptional level.

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Kinetic and Ultrastructural Studies of Interactions of Target-Sensitive Immunoliposomes with Herpes Simplex Virus†

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ABSTRACT: The bilayer phase of dioleoylphosphatidylethanolamine (PE) can be stabilized with palmitoyl-IgG monoclonal antibody to the glycoprotein gD of the herpes simplex virus (HSV). Interactions of PE immunoliposomes with the target virions were characterized by analyzing the kinetics of lipid mixing, by liposomal content release, and by ultrastructural studies. As revealed by a resonance energy transfer assay, lipid mixing between PE immunoliposomes and virions was very rapid, with a second-order rate constant (k_{app}) of 0.173 $(min)^{-1}$ $(\mu g/mL\ virus)^{-1}$. In comparison, content release from PE immunoliposomes was much slower and exhibited multiple-phase, mixed-order kinetics, indicating that liposome destabilization involved fusion of liposomes with HSV. The extent and the apparent rate of liposome destabilization were strongly dependent on liposome concentration. This was evident by the fact that only one to two liposomes were destabilized by each virus particle at low liposome concentration (0.1 μM). For higher liposome concentrations (1-10 μM), this value was 35-104. This finding implies that collision among the virus-bound liposomes is essential for the eventual collapse of PE immunoliposomes to form the hexagonal (H_{II}) equilibrium phase which was observed using freeze-fracture electron microscopy. Studies employing soluble gD, immobilized on latex beads, indicated that a multivalent antigen source is essential for PE immunoliposome destabilization. Immediately after liposome-virus binding, fusion of liposome with the viral membrane then follows. Upon growth of the fusion complexes, which increase to 35-104 liposomes for each virus, an eventual collapse of the structure results, driving PE to its equilibrium structure of H_{II} phase.

We have recently reported preparation of novel, antibody-coated phosphatidylethanolamine (PE)[1] liposomes that can be readily destabilized by binding to target membranes expressing the antigen [target-sensitive immunoliposome (Ho et al., 1986a,b)]. Target-sensitive (TS) immunoliposomes were constructed by employing palmitoyl-IgG (pIgG) antibody against glycoprotein D (gD) of herpes simplex virus (HSV) to stabilize a PE bilayer (Ho et al., 1986a). By itself, PE assumes a nonbilayer, hexagonal (H_{II}) structure under physiological conditions [see Cullis and DeKruijff (1979), Siegel (1987), Isrealachvili et al. (1980), and Verkleij (1984) for reviews]. Using both intact virions and viral antigen expressing cells, we have shown that a specific interaction exists between TS immunoliposomes and immobilized, multivalent antigen gD which results in the destabilization of the liposomes. This was readily detected by the release of liposome-entrapped calcein and further demonstrated by using antiviral drugs (Ho et al., 1986a,b, 1987a,b).

Since all of the reported measurements were done at equilibrium or near the end point of the destabilization process (i.e., 30 min after liposome-virus mixing), the nature of the intermediate steps remains to be elucidated. Because the understanding of such molecular events is crucial to optimize TS immunoliposome-mediated antiviral drug delivery and to optimize the TS immunoliposome-based immune detection assay, we have further investigated the interactions of TS immunoliposomes with target HSV virions. In this report, we elucidate the mechanism of TS immunoliposome and HSV-gD interactions on the basis of kinetic studies of lipid mixing, liposomal content release, and ultrastructural studies of intermediates. The results showed that binding of TS immunoliposomes to target HSV is characterized by fast lipid mixing, fusion between liposome and virus, and a subsequent slower, multiple-phase content release, eventually leading to

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[1] Abbreviations: PE, phosphatidylethanolamine; TS, target sensitive; pIgG, palmitoyl-IgG; gD, glycoprotein D; HSV, herpes simplex virus; DOPE, dioleoyl-PE; TPE, transphosphatidylated PE (from egg PC); PC, phosphatidylcholine; NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-PE; Rh-PE, *N*-(lissamine rhodamine B sulfonyl)-PE; EDCI, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; DOC, deoxycholate; PBS, phosphate-buffered saline; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; BSA, bovine serum albumin.

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