Carnitine palmitoyltransferase activity is present and high in the muscle and liver of lampreys (Agnatha)

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Insulin antagonism of dexamethasone induction of tyrosine aminotransferase in cultured fetal hepatocytes

A correlation between enzyme activity, synthesis, level of messenger RNA and transcription

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Previous studies have shown that insulin depresses the induction of tyrosine aminotransferase by glucocorticoids in cultured fetal rat hepatocytes. However, the site at which this inhibitory effect is exerted was not elucidated, since only enzyme activity was determined in such studies. Therefore, the effect of insulin on tyrosine aminotransferase synthesis, the level of its mRNA as well as the rate of transcription of the gene in isolated nuclei have been determined. The results obtained indicate that in cultures exposed to dexamethasone, Bt_2cAMP, insulin and combinations of these additives, there is an excellent correlation between the enzyme activity, enzyme synthesis and the level of mRNA. Run-on transcription experiments indicate that the reduction in the level of mRNA by insulin in dexamethasone-supplemented cultures is the result of a diminished rate of gene transcription.

Glucocorticoids and insulin have been implicated as important regulators in the development of several different tissues. In some systems shown to be glucocorticoid sensitive, it has been found that insulin augments the steroid response. For instance, both hormones are required to enhance glycogen synthetase activity in liver [1] and both are implicated in the differentiation of mammary epithelial cells and subsequent synthesis of α-lactalbumin and casein [2]. However, in many of the systems studied so far, the action of glucocorticoids is antagonized by insulin [3–6].

Although absent from fetal liver, tyrosine aminotransferase activity develops within a few hours after birth [7, 8]. Glucocorticoids have been implicated in this process, as adrenalectomy after birth largely prevents acquisition of the enzyme [7]. However, administration of glucocorticoids *in utero* fails to induce tyrosine aminotransferase prematurely [7, 9]. Paradoxically, these hormones can induce the enzyme in cultured fetal hepatocytes [10] which indicates that the cells are capable of synthesizing tyrosine aminotransferase and suggests that a factor which suppresses the glucocorticoid response is present in the *in utero* environment. Given that insulin levels are high in the fetus and decline postnatally [11, 12], and that it has been demonstrated in other systems to antagonize the effects of glucocorticoids, we reasoned that insulin might be this factor.

Subsequent studies with cultured fetal rat hepatocytes revealed that physiological concentrations of insulin markedly depress the induction of tyrosine aminotransferase by glucocorticoids [13, 14]. Furthermore, if the fetal plasma insulin concentration is artificially decreased by the administration of streptozotocin or anti-insulin serum, tyrosine aminotransferase is precociously induced by the endogenous flux of corticosterone that precedes birth [15]. Thus, there is good evidence that the natural decline in insulin which occurs at birth is a major contributing factor to the postnatal appearance of tyrosine aminotransferase.

It has been previously shown that the effects of glucocorticoids on tyrosine aminotransferase are mediated via enhanced levels of mRNA [16, 17] leading to increased synthesis of the enzyme [18, 19]. However, since the inhibitory effect of insulin has only been demonstrated using enzyme activity measurements, it cannot be assumed that it affects mRNA levels. For this reason, we have carried out a more detailed study of the effects of insulin and shown that the diminished activity of tyrosine aminotransferase is the result of decreased synthesis of enzyme which is directly due to lower levels of mRNA.

MATERIALS AND METHODS

Animals

The Wistar strain of the albino rat, *Rattus norvegicus*, was used. Fetal rats were obtained by timed pregnancy and are accurate to within 8 h. Term occurs at 22 days gestation in this colony.

Chemicals

Dexamethasone, bovine serum albumin, pyridoxal 5′-phosphate, 2-oxoglutarate, 2-mercaptoethanol, dithiothrei-

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Abbreviations. Bt_2cAMP, N^6,2′-*O*-dibutyryladenosine 3′,5′-phosphate; SSC, saline sodium citrate.

Enzymes. Aspartate aminotransferase (EC 2.6.1.1); glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); glycogen synthetase (EC 2.4.1.11); phosphorylase kinase (EC 2.7.1.38); tyrosine aminotransferase (EC 2.6.1.5).

tol, L-leucine, heparin, salmon-sperm DNA and Bt_2cAMP were supplied by Sigma Chemical Co., St Louis, MO, USA. Insulin was purchased as a concentrated solution of 100 units · ml^{-1} from Commonwealth Serum Laboratories, Melbourne, Australia. Nick translation reagents were obtained from International Biotechnologies Inc., New Haven, CT, USA. [^{32}P]dCTP, [α-^{32}P]UTP, L-[3,5-^{3}H]tyrosine, L-[3,4,5-^{3}H]leucine, Hybond-N and NCS tissue solubilizer are products of Amersham International plc, Bucks, UK. Zeta-Probe membrane, acrylamide and bisacrylamide were purchased from Bio-Rad Laboratories, Richmond, VA, USA. Fungizone, penicillin, streptomycin and glutamine were purchased from Grand Island Biological Co., Grand Island, NY, USA. Collagenase, ATP, GTP, CTP, UTP and proteinase K were obtained from Boehringer Mannheim, FRG. Fetal bovine serum and Eagle's minimal essential medium were purchased from Flow Laboratories, Sydney, Australia. Coomassie G-250 was obtained from Eastman Kodak Co., Rochester, NY, USA. Diethyldithiocarbamate and L-tyrosine were supplied by Merck, Darmstadt, FRG. Carboxymethyl-Sephadex C-50 was from Pharmacia Fine Chemicals, Uppsala, Sweden and sodium dodecylsulphate was from Pierce Chemical Co., Rockford, IL, USA. *Staphylococcus aureus* protein-A bacterial absorbent was supplied by Miles Yeda Ltd, Kiryat Weizmann, Rehovet, Israel. Nonidet P-40 was provided by Rampire Laboratories Pty. Ltd, Perth, Australia. RNasin and RQ1 DNase were purchased from Promega Biotec, Madison, WI, USA and tRNA was from Bethesda Research Laboratories, Gaithersburg, MD, USA.

Hepatocyte cultures

Cultures of fetal hepatocytes were prepared as previously described [10]. Briefly, livers derived from 19-day gestation rats were aseptically removed and diced using a Mickle chopper (Mickle Laboratories Engineering Co., Surrey, UK) fitted with a razor blade. The preparation was then incubated at 37 °C with collagenase (0.5 mg · ml^{-1} in Hank's balanced salts solution). After 15 min the mixture was filtered through cheesecloth and the suspension of cells washed twice with balanced salts solution by centrifugation at $50 \times g$ for 1 min and finally suspended in culture medium containing 10% charcoal-treated fetal bovine serum.

Cells were plated on collagen-coated 90-mm plastic culture dishes (Sterilin Products, Teddington, Middlesex, UK) in 10 ml medium and maintained at 37 °C in a water-saturated atmosphere of 95% air/5% CO_2. The following day, the cultures were washed with Hank's balanced salts solution to remove non-adherent cells which consisted mainly of erythroid cells and some non-viable cells. Medium was replaced daily. Hepatocytes isolated from six fetuses were plated onto each dish. This procedure gave a cell density on day 1 of approximately 4×10^6 cells/dish. Trypan blue exclusion test verified that more than 99% of the cells were viable at the time of assay.

Additions to cultures

Bt_2cAMP was dissolved in Hank's balanced salts solution at a concentration of 50 mM and sterilized by filtration through a 0.22-μm filter. This was diluted 1:100 to give a final concentration of 0.5 mM in culture medium. Control cultures received an equal volume of salts solution. Dexamethasone was prepared as a 10 μM stock solution in 1,2-propanediol and diluted 1:1000 to give a final concentration of 10 nM in culture medium. Control cultures received an equal volume of 1,2-propanediol. Insulin was added to give a final concentration of 100 nM by diluting a concentrated stock solution of 100 units · ml^{-1}.

Tyrosine aminotransferase assay

Preparation of cytosol and tyrosine aminotransferase assay were carried out as described previously [10]. The procedure used involves ion-exchange chromatography of each cytosolic extract in order to minimize the amount of aspartate aminotransferase which is present at very high levels in fetal liver extracts. At these levels, the amount of tyrosine transaminated by aspartate aminotransferase would have interfered with the assay which measures the quantity of [^{3}H]tyrosine which is transaminated [10]. The protein content of cytosolic extracts was determined using the dye-binding method of Bradford [20] using bovine serum albumin as a standard.

Tyrosine aminotransferase synthesis

After 48 h of culture, including an exposure period to the indicated hormones, fetal hepatocytes were incubated a further 45 min in 4.5 ml leucine-free medium containing 250 nM L-[3,4,5-^{3}H]leucine (12.5 μCi · ml^{-1}). It has been established that the rate of incorporation of labelled leucine into both tyrosine aminotransferase and total cytosolic protein is linear for at least 45 min. This is probably because this medium contains 10% charcoal-treated fetal bovine serum which would be a source of additional leucine. At the end of the incubation, cultures were washed extensively with cold balanced salts solution [21], harvested and suspended in 1.5 vol. (mass/vol.) buffer A (150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.2) containing 2 mM leucine, 0.02% sodium azide and 0.05% Nonidet P-40. The suspension was sonicated for 15 s in a Branson model B-12 sonifier (Branson Sonic Power Co., CN, USA) at 2 A using a microtip and the extract was centrifuged in a Beckman airfuge for 15 min at $165000 \times g_{max}$ at 4 °C to prepare cytosol. A 10-μl aliquot was used in the estimation of total cytosolic protein synthesis as described by Mans and Novelli [22].

Prior to incubating the extract with anti-(tyrosine aminotransferase) serum, the cytosol was incubated with an equal volume of 10% protein-A bacterial absorbent suspended in buffer A containing 0.05% Nonidet P-40 and 1% bovine serum albumin for 15 min at 25 °C with regular mixing. This pretreatment of the cytosol was essential for minimizing non-specific binding of either leucine or labelled protein to protein-A bacterial absorbent after exposure to antibody. After centrifugation at $2000 \times g$ for 20 min at 4 °C, aliquots of the particle-free supernatant were incubated with either 5 μl of a polyclonal antibody specific for tyrosine aminotransferase or an equivalent volume of non-immune rabbit serum for 15 min at 25 °C. The equivalence point for the titre of the antiserum was 57.6 mU · μl^{-1} rabbit serum, and thus the volume of cytosol incubated was optimized for each hormonal treatment. After a 15-min incubation with rabbit serum, immunocomplexes were recovered by adsorption to protein-A bacterial absorbent by addition of 10 μl of a 10% solution of the adsorbent and incubating for a further 15 min at 25 °C. The precipitate was collected and washed three times with 1.5 ml buffer A containing 0.05% Nonidet P-40 and 1% bovine serum albumin by centrifugation at $2000 \times g$ for 15 min at 4 °C.

The precipitated complex was heated at 90 °C for 10 min in 80 µl 62 mM Tris, pH 6.8 containing 5% 2-mercaptoethanol, 2% SDS, 10% glycerol and 0.01% bromophenol blue, and the supernatant was subjected to SDS/polyacrylamide gel electrophoresis according to the procedure of Laemmli [23] in which the resolving and stacking gels contained 10% and 3% acrylamide, respectively. On completion of electrophoresis, the gels were removed, frozen at −20 °C and then cut into 2-mm slices using a Hoefer model DE 113 gel slicer. The slices were solubilized in 10 ml of a scintillant containing 5.28 g 2,5-diphenyloxazole, 120 ml NCS tissue solubilizer and 3.6 ml of NH_4OH in 880 ml of toluene. After dark-adapting samples for at least 48 h, radioactivity was determined in a Beckman scintillation counter. The level of radioactivity specifically incorporated into tyrosine aminotransferase was determined by subtracting non-specific incorporation (non-immune serum) from total (immune serum) and is expressed as a percentage of the amount of radioactivity incorporated into all cytosolic proteins.

Isolation of total RNA and determination of tyrosine aminotransferase mRNA levels

Total RNA was isolated from liver tissue or cell cultures according to the method of LeMeur et al. [24]. The samples were dispersed in 5 ml buffer containing 3 M LiCl, 6 M urea, 10 mM sodium acetate (pH 5.0), 200 µg · ml^{-1} heparin and 0.1% SDS using an Ultra-turrax and then left overnight at 4 °C. The precipitated RNA was collected by centrifugation at 15000 × *g* for 20 min, washed four times with 4 M LiCl, 8 M urea in water, dissolved in 50 mM sodium acetate (pH 5.0) in 1% SDS and extracted with phenol/chloroform and ethanol-precipitated. The RNA was finally dissolved in water and its concentration was estimated by measuring the absorbance at 260 nm. For dot blotting 2.5 µg, 5 µg and 10 µg RNA were applied to a Bio-Rad Zeta-Probe nylon membrane. For Northern analysis, 25 µg RNA was electrophoresed according to the method of Lehrach et al. [25] and then electrophoretically transferred to Hybond-N using a Bio-Rad Transblot apparatus.

Tyrosine aminotransferase mRNA was detected by hybridization to a ^{32}P-labelled probe pcTAT3 [26] and visualization of the radioactivity by autoradiography. The cDNA probe was labelled by nick translation using reagents obtained from International Biotechnologies Inc. Hybridization in 50% deionized formamide, 5 × SSC, 0.1% SDS, 5 × Denhardt's solution and 250 µg · ml^{-1} sonicated salmon-sperm DNA was performed at 43 °C for 36 h after 5 h of prehybridization. This was followed by washing according to the protocol outlined in the instructions accompanying the respective membranes. The intensity of the image produced on X-ray film was quantified using a Bio-Rad model 620 video densitometer and is expressed as a percentage of the level observed in hepatocytes exposed to both Bt_2cAMP and dexamethasone. It was established that, under these conditions, linearity between counts/min (determined by liquid scintillation counting of the membrane) and peak area was achieved up to 450 cpm. The use of X-ray films with different sensitivities ensured that all data fell within this linear range.

Run-on transcription assay

Nuclei were isolated from cultured hepatocytes by the method of Becker et al. [27], suspended in 60% glycerol, 4 mM $MnCl_2$, 1 mM $MgCl_2$, 5 mM dithiothreitol, 20 mM Tris, pH 7.4 and stored at −20 °C. Transcription of tyrosine aminotransferase was performed as described by Danesch et al. [28] with the following modifications. Isolated nuclei ($5-10 \times 10^6$) were incubated in a reaction mixture containing 50 mM Hepes, pH 8.0, 150 mM NH_4Cl, 1 mg · ml^{-1} bovine serum albumin, 1 mM $MnCl_2$, 12.5% glycerol, 3.5 mM $MgCl_2$, 0.25 mM dithiothreitol, 1 µl (60 U) RNasin, 0.5 mM ATP, GTP, CTP and UTP, 100 µCi [α-^{32}P]UTP (3000 Ci · $mmol^{-1}$) for 20 min at 25 °C. After the mixture was adjusted to final concentrations of 5 mM Tris, pH 7.5, 5 mM $MgCl_2$ and 5 mM $CaCl_2$, 1 unit RQl DNase was added and the mixture was incubated for 5 min at 37 °C. Then it was treated with proteinase K (150 µg · ml^{-1}) at 37 °C for 30 min, and RNA was isolated by phenol/chloroform extraction followed by trichloroacetic acid precipitation and ethanol precipitation in the presence of 0.05 mg · ml^{-1} tRNA. After treating again with RQl DNase as above, RNA was isolated by phenol/chloroform extraction, collected by ethanol precipitation as above and hybridized at 37 °C for 72 h in 55% formamide, 4 × SSC, 0.1 M sodium phosphate, pH 6.8, 1 × Denhardt's solution, 0.1% SDS, 100 µg · ml^{-1} salmon-sperm DNA and 10% dextran sulphate to 0.25 pmol of each of the following nitrocellulose-bound DNA: (a) tyrosine aminotransferase genomic subclones TAT EH 0.95, TAT EH 2.45, TAT EE 1.05 [29]; (b) albumin genomic subclones B, C and D [30]; (c) cDNA pGAPDH-13 [31]; (d) pUC8. Filters were washed at 65 °C three times in 1 × SSC, 0.1% SDS (15 min/wash), and then twice in 0.1 × SSC, 0.1% SDS (45 min/wash) and associated radiolabel was visualized by fluorography and assessed by optical densitometry using a Bio-Rad video densitometer, model 620 (Bio-Rad Laboratories, Richmond, CA, USA). Data was corrected for non-specific binding to pUC8 and

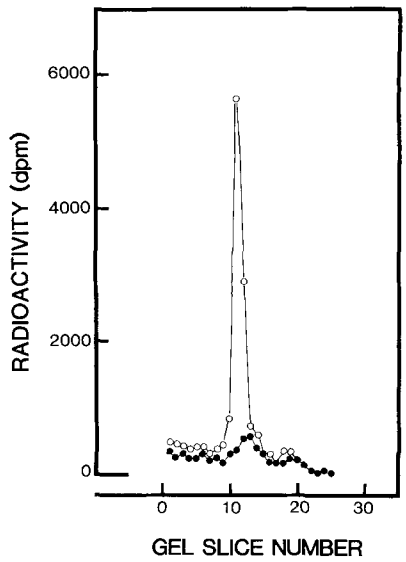


Fig. 1. *Specificity of immunoprecipitation of tyrosine aminotransferase in extracts from cultured fetal rat hepatocytes.* Immunoprecipitates were prepared from extracts derived from 19-day fetal hepatocyte cultures as described in Materials and Methods. Cultures were maintained in medium containing 0.5 mM Bt_2cAMP for 32 h, then supplemented with 10 nM dexamethasone for a further 16 h and exposed to L-[^3H]leucine for 45 min before extracts were prepared. Extracts were incubated with either immune (○——○) or non-immune (●——●) rabbit serum. Radioactivity recovered from each gel slice after electrophoresis is presented

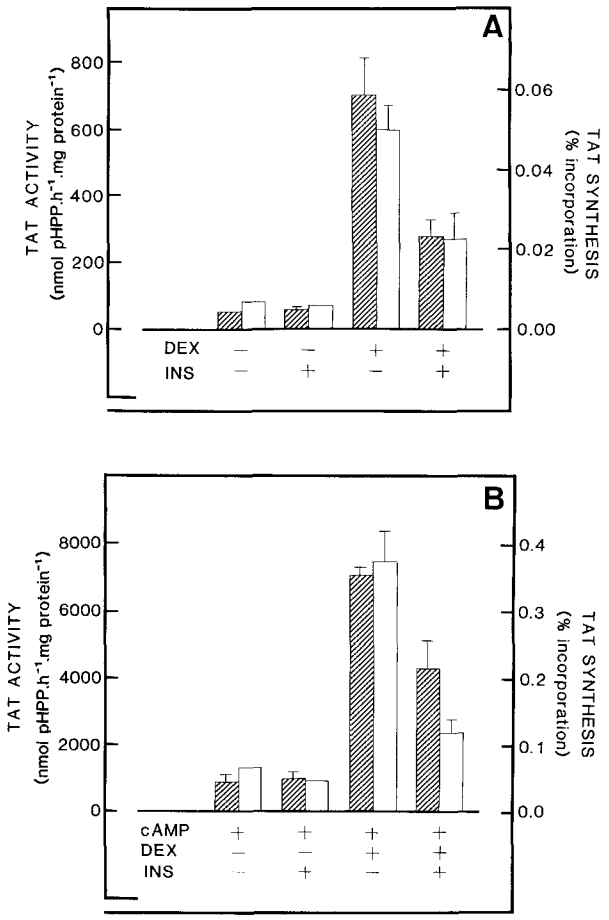


Fig. 2. *Effect of insulin on induction of tyrosine aminotransferase synthesis in the absence (A) and presence (B) of Bt_2cAMP.* Immunoprecipitates were prepared from extracts derived from 19-day fetal hepatocyte cultures as described in Materials and Methods. Cultures were maintained in standard medium, or medium containing 0.5 mM Bt_2cAMP (cAMP), or 100 nM insulin (INS) or both additives (as indicated) for 32 h, then supplemented with 10 nM dexamethasone (DEX) or an appropriate volume of 1,2-propanediol for a further 16 h. The cells were then exposed to L-[^{3}H]leucine for 45 min before extracts were prepared. Synthesis (open bars) is presented as the mean ± SEM of at least three cell preparations and expressed as percentage incorporation of radioactivity into tyrosine aminotransferase. This is compared with previously published data [14] on tyrosine aminotransferase activity (shaded bars); pHPP, *p*-hydroxyphenylpyruvate; TAT, tyrosine aminotransferase

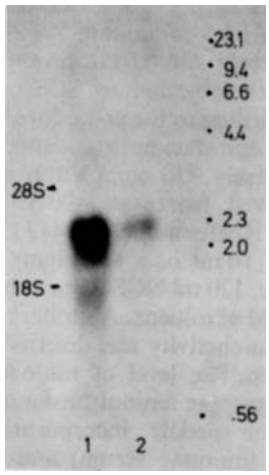


Fig. 3. *Northern blot analysis of tyrosine aminotransferase mRNA.* Total RNA was extracted from hepatocyte cultures and electrophoresed and transferred to Hybond-N as described in Materials and Methods. The membrane was probed with [^{32}P]cDNA specific for tyrosine aminotransferase and processed for autoradiography. 28S and 18S ribosomal RNA, as well as *Hin*dIII-digested λ DNA marker positions are indicated. Lane 1, 20 μg RNA isolated from hepatocyte cultures treated with dexamethasone and Bt_2cAMP; lane 2, 20 μg RNA from control cultures

expressed relative to transcription of the glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

Tyrosine aminotransferase synthesis

Fig. 1 shows a typical gel analysis of an immunoprecipitate obtained from reacting anti-(rat tyrosine aminotransferase) serum with extracts from cultured fetal hepatocytes exposed to L-[3,4,5-^{3}H]leucine. A single peak of radioactivity labelled protein at a position corresponding to M_r 53000 is obtained. Also shown in the figure is non-specific incorporation, which unless corrected for, would lead to an overestimation of tyrosine aminotransferase synthesis especially where low levels of incorporation are observed. Therefore, all results represent the specific component of incorporation i.e. total incorporation (immune serum) minus non-specific incorporation (non-immune serum).

Fig. 2A summarizes the effects of dexamethasone, insulin and dexamethasone together with insulin on tyrosine aminotransferase synthesis and compares it with previously published activity data [14]. Upon exposure to 10 nM dexamethasone, synthesis (measured as the incorporation of [^{3}H]leucine into tyrosine aminotransferase) is increased by sevenfold. When the cells are cultured in media containing 100 nM insulin there is no effect on enzyme activity or synthesis. However, the presence of insulin markedly reduced the response of the cells to dexamethasone, producing a 65% ($P < 0.01$) and 61% ($P < 0.01$) reduction in activity and synthesis, respectively. The results from experiments designed to test the effects of dexamethasone and insulin individually or in combination in the presence of 0.5 mM Bt_2cAMP are presented in Fig. 2B. Bt_2cAMP alone induces enzyme activity by approximately 16-fold and synthesis by ninefold. Insulin has no effect on the induction of activity and synthesis by Bt_2cAMP. In combination, Bt_2cAMP and dexamethasone result in a synergistic induction of tyrosine aminotransferase of 120-fold in terms of activity and 55-fold in terms of synthesis. The presence of insulin, reduces the induction of enzyme activity by dexamethasone and Bt_2cAMP by 43% ($P < 0.02$). Its effect in terms of synthesis is a 68% reduction ($P < 0.001$). Overall, the results presented in Fig. 2 show a very good correlation between enzyme activity and synthesis ($r = 0.945$).

Tyrosine aminotransferase mRNA

Northern blot analysis of tyrosine aminotransferase mRNA in total RNA extracts reveals that under the conditions of hybridization and washing used in this study a single species of mRNA is detected by the [^{32}P]cDNA probe

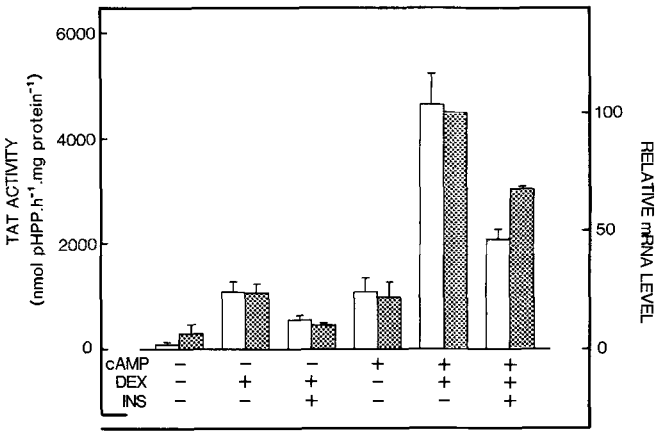
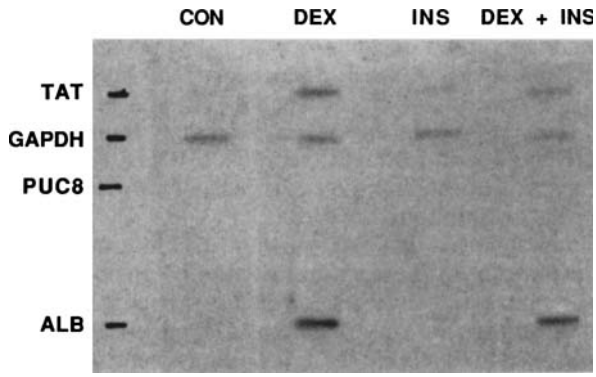


Fig. 4. *Correlation between tyrosine aminotransferase activity and mRNA level in cultured hepatocytes.* Total RNA was extracted from 19-day fetal hepatocyte cultures and tyrosine aminotransferase mRNA (shaded bars) detected as described in Materials and Methods. Enzyme activity (open bars) was determined in duplicate cultures. Cultures were maintained in standard medium, or medium containing 0.5 mM Bt_2cAMP (cAMP), or 100 nM insulin (INS) or both additives for 32 h, then supplemented with 10 nM dexamethasone (DEX) or an appropriate volume of 1,2-propanediol for a further 16 h. The results represent the mean ± SEM of three separate cell preparations; TAT, tyrosine aminotransferase; pHPP, *p*-hydroxyphenylpyruvate



	CON	DEX	INS	DEX + INS
TAT	0.07	0.48	0.04	0.11
GAPDH	0.38	0.30	0.25	0.13
PUC8	0.06	0.04	0.03	0.06
ALB	0.07	0.89	0.04	0.56
TAT/GAPDH	0.03	1.69	0.05	0.71
ALB/GAPDH	0.03	3.27	0.05	7.14

Fig. 5. *Nuclear run-on transcription analysis for tyrosine aminotransferase in cultured hepatocytes.* Hybridization of ^{32}P-labelled mRNA transcribed by nuclei isolated from control cultures (CON), and cultures treated with dexamethasone (DEX), insulin (INS) or a combination of dexamethasone and insulin (DEX/INS) to cDNA coding for tyrosine aminotransferase (TAT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), albumin (ALB) and to pUC8 (PUC8) was determined as described in Materials and Methods. The signals were quantified by densitometry (shown in the table as relative absorbance) and expressed relative to the GAPDH signal after subtraction of the signal obtained for pUC8

for tyrosine aminotransferase (Fig. 3). Lane 1 shows a substantial induction in the level of mRNA in cultures exposed to a combination of dexamethasone and Bt_2cAMP compared with controls. The size of the mRNA is judged to be about 2250 bases in comparison to the size markers. These conditions were subsequently used for quantification of mRNA levels in total RNA extracts using dot blots.

Fig. 4 summarizes data where both activity and levels of tyrosine aminotransferase mRNA were measured in replicate cultures in three separate experiments designed to test the effect of insulin on dexamethasone in the absence and presence of Bt_2cAMP. The decrease in induction of tyrosine aminotransferase activity by glucocorticoids in the presence of 100 nM insulin is accompanied by a corresponding decrease in the level of its mRNA (80% inhibition, $P < 0.05$). In the presence of Bt_2cAMP, the response to steroid is greatly enhanced, i.e. synergism is observed at the level of tyrosine aminotransferase mRNA. Insulin remains effective in inhibiting the response to steroid under these conditions (41% inhibition, $P < 0.001$). It was not possible to include insulin-containing controls in these experiments in which both mRNA and activity measurements were made. This would have required more timed-pregnant rats than could be obtained on any one day. Thus, in each instance, percentage inhibition has been determined relative to the respective control without insulin. This can be justified on the basis that the addition of insulin to control cultures or cultures containing Bt_2cAMP has no effect on tyrosine aminotransferase activity or synthesis. Under all conditions a good correlation is observed between the level of enzyme activity and the level of its corresponding mRNA ($r = 0.967$).

Nuclear run-on experiments (Fig. 5) reveal that transcription of the *TAT* gene is barely detectable in control cultures and it is substantially increased in cells exposed to dexamethasone. When insulin is also present, the increase caused by dexamethasone is inhibited by 60%. In contrast no such effect is observed when transcription of the albumin gene is examined in nuclei isolated from these cells. In fact the data suggests that insulin augments the dexamethasone induction of transcription of the albumin gene. All transcription is expressed relative to transcription of the glyceraldehyde-3-phosphate-dehydrogenase gene which is recognized as a gene which is constitutively expressed in liver cells.

DISCUSSION

Liver enzymes appear at different stages of development [32], suggesting that a combination of factors is likely to be responsible for the various stages of enzymic differentiation of liver. For a particular gene to be expressed, several conditions must be satisfied. Not only must the gene be accessible to RNA polymerase, but the appropriate humoral conditions must prevail in order that transcription is initiated at a significant level. Subsequently, the initial transcript must undergo post-transcriptional processing before it can be translated into protein. Furthermore, in the case of an enzyme, it may be necessary to activate the protein before it is capable of catalyzing a biological reaction.

In the case of tyrosine aminotransferase, several lines of evidence suggest that the postnatal appearance of enzymic activity in the liver does not coincide with the acquisition of RNA polymerase accessibility to the gene to facilitate its transcription. For instance, the enzyme can be precociously induced *in utero* by administration of glucagon [32] and

specific tyrosine aminotransferase activity has been demonstrated in cultured explants of fetal liver [33] and in cultured fetal hepatocytes exposed to glucocorticoids [10]. Since there is a pre-term flux of glucocorticoid [34], and because glucocorticoids induce the enzyme in postnatal rat liver [7, 9, 18, 19], it has been difficult, until recently, to explain the postnatal appearance of the enzyme or its lack of response to glucocorticoids administered *in utero*.

Previously, we have shown that insulin antagonizes the induction of tyrosine aminotransferase by glucocorticoids in fetal hepatocyte cultures [13, 14]. Furthermore, administration of anti-insulin serum to fetuses, or artificial depression of the insulin levels in the fetus using streptozotocin, precociously induces tyrosine aminotransferase [15]. Since insulin levels are markedly elevated in the fetus and decline at birth [11, 12], it appears likely that insulin is an important regulator of tyrosine aminotransferase development in the liver. The experiments described in this communication were designed to determine the level at which this regulation is exerted.

Since previous reports [13, 14] were based on enzymic activity alone, we were unable to exclude the possibility that insulin was exerting its effect by modifying the catalytic activity of tyrosine aminotransferase. Indeed, insulin has been shown to enhance the activity of glycogen synthetase [1] and, conversely, inactivate phosphorylase kinase [35] by this mechanism. In the experiments reported in this communication, replicate cultures were established so that enzyme activity and either enzyme synthesis or tyrosine aminotransferase mRNA levels could be determined. The data shows that all three parameters correlate extremely closely. This is true for the enhanced level of expression observed in cultures treated with dexamethasone, the synergistic response elicited by the simultaneous administration of dexamethasone and Bt_2cAMP, as well as the diminution in response in both instances when insulin is also present. The effectiveness of insulin in both these situations demonstrates that it is unlikely to be mediated via an inhibition of the phosphorylation cascade pathway since cAMP is unable to reverse its inhibitory effect. Collectively, these results lead to the conclusion that in fetal hepatocytes insulin acts by reducing the level of mRNA for tyrosine aminotransferase and that this in turn results in a lower rate of enzyme synthesis. This contrasts with the situation in the adult rat liver where insulin has been shown to promote increased levels of mRNA coding for tyrosine aminotransferase [36], and in HTC cells where it stabilizes the enzyme against degradation [37].

The diminution in mRNA levels in fetal hepatocytes maintained in dexamethasone-supplemented medium in response to insulin could be the result of an increase in the rate of mRNA degradation or a decrease in the rate of its synthesis. The run-on transcription experiment shows that the latter is more likely since transcription rates in nuclei isolated from cultures treated with dexamethasone are considerably higher than that observed in cultures treated with both dexamethasone and insulin. It should be emphasized that this phenomenon is only observed with tyrosine aminotransferase, as transcription of albumin in the same nuclei is not reduced, but in fact enhanced, by this combination of hormones. As transcription activities are also expressed relative to that observed with glyceraldehyde-3-phosphate dehydrogenase, the effect on tyrosine aminotransferase appears to be specific.

In conclusion, these results support the view that, in fetal hepatocytes, insulin is a negative regulator of tyrosine aminotransferase expression. It acts in opposition to the inductive effect of glucocorticoids and thus the high levels of insulin in the fetus may explain why the enzyme is not normally observed in the fetal liver and appears postnatally when insulin levels decline.

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