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PROPERTIES AND HORMONAL REGULATION OF HEPATIC TYROSINE AMINOTRANSFERASE OF VERTEBRATES

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INTRODUCTION

The first reaction in the catabolism of tyrosine in mammals and higher vertebrates is the transfer of its amino group to 2-oxoglutarate (1). This reaction which occurs primarily in the liver is the rate-limiting step in the sequence (2) and is catalyzed by tyrosine aminotransferase (TAT) (EC 2.6.1.5).

TAT has an absolute specificity for 2-oxoglutarate and under comparable conditions oxaloacetate and pyruvate are not utilized to any significant extent (3). The enzyme has a higher affinity for pyridoxal 5'-phosphate, its coenzyme, than for tyrosine and 2-oxoglutarate (4,5). Unlike other aminotransferases, TAT appears to be easily resolved of pyriodoxal 5'-phosphate with the result that the coenzyme behaves like a true substrate in the transamination reaction (6). The enzyme requires sulfhydryl groups for catalytic activity and a reactive thiol group is in or near its active site (7, 8).

Several papers dealing with the purification of the enzyme from a variety of sources (mammals, aves, amphibia, fungi and trypanosomes) have been published (9 - 15). Its molecular weight has been determined and is aboyut 100,000 (14, 16-18). TAT is structurally a dimer, consisting of 2 subunits which appear to be identical in size as well as charge (14, 17, 18). The subunits are the immediate product of translation and are complexed to form the dimeric molecule typical of the functional enzyme (18). TAT plays an important role in metabolism in providing carbon skeletons in the forms of fumarate and acetoacetate for gluconeogenesis and ketogenesis respectively as well as in the regulation of concentrations of tyrosine in tissues and blood (19, 20).

Hepatic TAT of higher vertebrates is induced by a range of hormonal and nutritional influences. This property makes TAT a very suitable enzyme for studying the regulation of protein synthesis. The induction of the enzyme occurs in a variety of systems such as in vivo, in perfused liver, in hepatomas and in isolated hepatocytes (21). The effects of glucocorticosteroids, glucagon or cAMP and insulin on enzyme induction have been extensively studied in order to indicate the integration of metabolic responses provoked by hormonal interactions at molecular level.

TAT has a very short half-life of the order of 1.5h, whereas the half-life of the average protein in rat liver has been reported in different studies to be between 2 and 3 days (22). This unique property indicates that TAT, in addition to its use as a model system to investigate gene expression in animals, can also serve as a valuable system for the study of intracellular protein degradation (23, 24), an area of biochemistry that is one of the least understood.

MOLECULAR PROPERTIES

Isoenzymes of TAT

TAT activity is found in the sytosol and in the mitochondria (25). Both forms are present in liver, heart, kidney, skeletal muscle and brain. The distribution of the two enzymes between cytoplasm and mitochondria varies from tissues to tissue.

The substrate specificities and kinetic properties of the two forms of TAT are quite different. The mitochondrial enzyme can transaminate a wide range of amino acids and readily utilizes oxaloacetate and pyruvate in place of 2-oxoglutarate whereas the cytosol enzyme has a specific requirement for 2-oxoglutarate and shows negligible activity with other keto acids (26). Aspartate inhibits the mitochondrial enzyme non-competitively with respect to tyrosine (26). Cytosol TAT is not inhibited by aspartate in the concentration range where inhibition of the mitochondrial enzyme occurs. In addition, at neutral pH the cytosol enzyme is anionic while the mitochondrial form is cationic. The two forms of TAT have different pH optima and Michaelis constants for tyrosine (25, 26). Thus, the isoenzymes differ on the basis of charge, substrate specificity, aspartate inhibition and Km values for tyrosine, indicating that the two proteins are functionally independent enzymes whose biosyntheses are possibly governed by separate structural genes. In the liver, cytosol TAT predominates and is actively involved in the catabolism of tyrosine (25). The precise role of the mitochondrial enzyme is obscure. It could provide some special and emergency needs of the cell. One unique example has been reported where, in the absence of cytosol TAT activity in a patient, the mitochondrial enzyme operates at an increased rate (27). In kidney and brain, mitochondrial TAT is predominant. It has been suggested that when the enzyme is bound to the mitochondrial membrane it presents a lower affinity for tyrosine (27). Such a change would be especially important in the brain as the catabolic role of TAT has to be moderated since the internal pool of tyrosine controls catecholamine synthesis.

However, the existence of the two forms of TAT has been called to question (28). Aspartate is the best substrate of the mitochondrial enzyme and more than 70% of its activity can be precipitated by antibodies specific for mitochondrial aspartate aminotransferase (28). Thus, it is possible that mitochondrial TAT is identical to mitochondrial aspartate aminotransferase. The enzyme which transaminates tyrosine in the heart has a lot of properties in common with aspartate aminotransferase, suggesting that the two enzymes might be identical as well (29). There, are also reports of enzymes which transaminate tyrosine in adrenal and thyroid glands (30, 31). Again transamination of tyrosine in these tissues could be due to aspartate aminotransferase because of its broad substrate spectrum.

Multiple forms of cytosol TAT

Cytosol TAT can be separated into three molecular forms in vitro by chromatography on hydroxylapatite or CM-Sephadex C-50 (28, 32, 33) or by isoelectric focussing (34) with no change in total TAT activity. The three forms are immunologically identical and have similar kinetic and catalytic properties (33). There are significant variations in the proportions of these forms when isolated under different extraction conditions (35). The three forms have been separated and purified and are designated I, II and III in order of their elution from hydroxylapatite (36). They have different molecular weights and isoelectric points and form III is more basic than forms II and I. Form I is comprised of two 53,000-dalton monomers, form III has two 49,000-dalton monomers and form II contains one of each sized monomer (37).

The origin of the multiple forms had previously been attributed to carbamylation, phosphorylation and modification of free sulfhydryl groups (33, 35, 38). It has been demonstrated that none of these pocesses is directly involved in the interconversion ofthe forms (10). It is now established that a lysosomal factor converts the immediate translation product, form I, to the two secondary forms, II and III (10). The converting factor which is also called cathepsin T has been purified and characterized and it has the properties of a thiol proteinase (39). The proteolytic

characteristics of cathepsin T and the difference in molecular weights of the multiple forms indicate that they originate through limited proteolysis. Indeed, cathepsin T cleaves a peptide which has a molecular weight of about 4,500 from form I (40). The difference in size between forms I and III is thus accounted for. The amino acid composition of the cleaved peptide has been determined and is composed mainly of acidic amino acids (40) and accounts for the fact that form III has a more basic isoelectric point than form I.

The physiological significance of the multiple forms of cytosol TAT is not known although there is a suggestion that forms II and III are the immediate products in the early steps of degradation of form I (40).

Sub units of TAT

Hepatic TAT from mammalian and avian species has a molecular weight of about 100,000 (16, 17, 41). The enzyme is composed of two sub units (17, 18, 41). The sub units are apparently identical in size and charge. A single electrophoretic band is obtained after sodium dodecyl sulphate (SDS) denaturation and also when the enzyme is denatured and subjected to electrophoresis in 8M urea, conditions under which net charge contributes as a determinant of migration (18). The molecular weight of the enzyme sub unit has been determined by electrophoresis on SDS polyacrylamide gels and by centrifugation in sucrose gradients containing SDS. Both analyses yield values slightly greater than 50,000 which is about half the molecular weight of the functional enzyme (14, 18, 41). Four moles of pyridoxal 5'-phosphate are bound per mole of enzyme, two moles per subunit (42). Previous reports suggesting a tetrameric structure for TAT with subunit size of about 32,000 were based partially on coenzyme analyses which did not distinguish functional from non-functional coenzyme (16). Current evidence does not support a tetrameric structure and indicates that TAT is structurally dimeric and consists of two subunits which appear to be identical (18, 41).

CATALYTIC AND KINETIC PROPERTIES

Catalyzed reactions

In mammals, the liver is principal site of gluconeogenesis. Tyrosine is carbolized in this tissue ultimately to acetoacetate and fumarate by the sequential action of six enzymes (see Fig. 1). The carbon skeleton of tyrosine in the form of fumarate then enters the gluconeogenic pathway or tricarboxylic acid cycle. The first reaction in the breakdown of tyrosine involves the removal of its &-amino group and subsequent transfer to 2-oxoglutarate which is the sole amino group acceptor (1, 7). This transamination reaction which is the rate-limiting step in the sequence is catalyzed by TAT (1).

The net stoichiometry of the overall transamination reaction has been verified (43) and is represented as follows:

L-tyrosine + 2-oxoglutarate $\rightarrow$ L-glutamic acid + p-hydroxyphenylpyruvate (pHPP).

The reaction is reversible with the position of equilibrium in favour of the formation of glutamic acid and pHPp (43). The reverse reaction is inhibited by pHPP even at a very low concentrations.

TAT requires pyridoxal 5'-phosphate, its coenzyme, for catalytic activity. The enzyme is saturated with pyridoxal 5'-phosphate at very low levels of coenzyme (16, 18). TAT is almost completely reactivated by the addiction of pyridoxal 5'-phosphate to the resolved enzyme (16) suggesting that resolved TAT is not inactivated irreversibly. In contrast only a fractional restoration of aspartate aminotransferase activity occurs by the addition of pyridoxal 5'-phosphate to the resolved enzyme (43).

Cytosol TAT has a very narrow substrate spectrum and can transamiate only a limited number of subsrates, some of which include 3-iodo-tyrosine and 3,4-dihydroxyphenylalanine. The inhibitory effects of a variety of substitutions on the aromatic ring of the tyrosine molecule have been studied and the observations made can be summarized as follows:

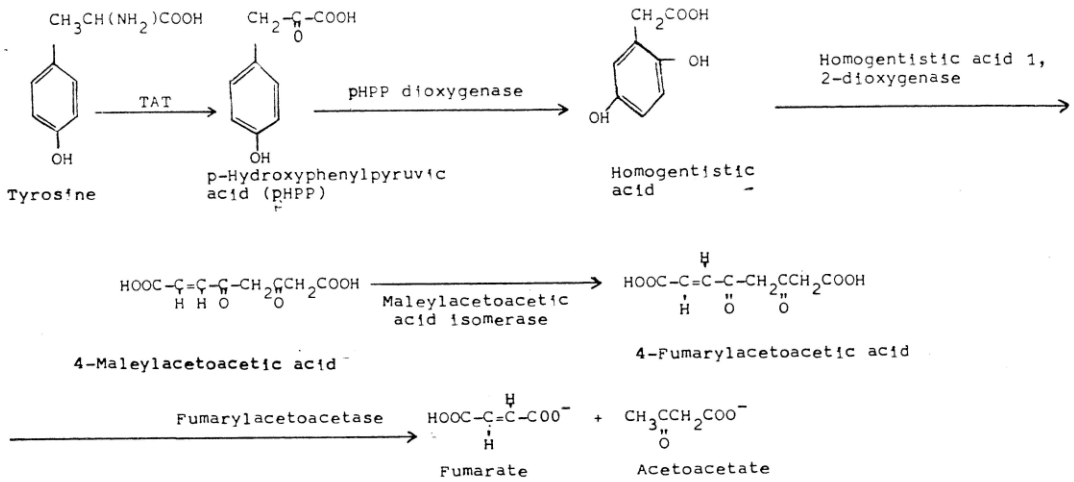


Fig. 1: Catabolism of tyrosine to fumarate and acetoacetate.

(i) Disubstitution results in greater inhibition than monosubstitution

(ii) Parasubstitution results in somewhat greater inhibition than does ortho- or metasubstitution.

(iii) The presence of a polar carboxyl group results in more effective inhibition than does a non-polar methyl group.

(iv) Of the parasubstituted benzoic acids, the following sequence is observed with respect to effectiveness as inhibitors:

$$-OH > -NH_2 > --COOH > -NO_2$$

Thus, the aromatic portion of tyrosine and the parahydroxyl configuration are necessary components of the aminotransferase system.

Mitochondrial TAT which has a very broad substrate spectrum tranaminates other ring-substituted phenylalanines and is responsible for most of the aromatic transaminating activity of the brain using 2-oxoglutarate, oxaloacetate or pyruvate as amino group acceptors (44). Mitochondrial TAT also transaminates m- tyrosine, p-chlorophenylalanine and p-fluorophenylalanine at rates of 16 - 25% that for tyrosine (3).

Information on the susceptibility to transamination of these substituted amino acids is important because several of them are of biological interest. m- Tyrosine can serve as a precursor of 3,4-dihydroxyphenylalanine and hence of the catecholamines. p-Chlorophenylalanine is an inhibitor of serotonin biosynthesis causing various pharmacological and behavioural effects in vivo (45) and p-fluorophenylalanine is an antimetabolite of phenylalanine and tyrosine, reported to have inhibited the growth of tumors in mice on a low phenylalanine diet (46). However, the metabolic fate and mode of action of these substances have not been completely delineated.

Catalytic mechanism

The transamination reaction catalyzed by TAT is an example of a double-displacement reaction and like other aminotransferases shows the corresponding ping-pong mechanism (16, 7).

Isotopic exchange occurs between tyrosine and pHPP, dependent only on enzyme and coenzyme but not on 2-oxoglutarate or glutamate (6). This exchange has the same initial rate as the complete transamination reaction catalyzed by TAT. In addition, there is a net chemical synthesis of pHPP from tyrosine in the absence of 2-oxoglutarate but presence of pyridoxal 5'-phosphate. These observations inicate a ping-pong mechanism similar to that found for other aminotransferases but with the difference that coenzyme can dissociate freely from TAT and therefore behaves like a true substrate in the transamination reaction (5, 6). Moreover, pyridoxal 5'-phosphate can combine with tyrosine to form a Schiff's base and the ping-pong mechanism exhibited by TAT is random in the sense that either coenzyme or Schiff's base of coenzyme and amino acid can add to the apoenzyme. In contrast, the coenzymes of most aminotransferases including aspartate aminotransferase and alanine aminotransferase do not dissociate (47) and in these enzymes pyridoxal 5'-phosphate acts as a 'shuttle' for the amino group.

All aminotransferases appear to have the same coenzyme, i.e. pyridoxal 5'-phosphate. The pyridoxal 5'-phosphate is usually non-covalently linked to the enzyme protein, presumably through the charged ring nitrogen atom and functions as a carrier of amino groups (47). During its catalytic cycle the coenzyme undergoes reversible transitions between its free aldehyde form, pyridoxal 5'-phosphate, and its form, pyridoxamine 5'-phosphate. In this cycle the unprotonated alpha-amino group of the amino donor is covalently bound to the carbon atom of the aldehyde group of the enzyme-bound pyridoxal 5'-phosphate withelimination of water to form an aldimine which tautomerizes to the corresponding ketimine. Both the aldimine and ketimine contain the C = N - structure characteristic of a Schiff's base. Addition of water leads to formation of free alpha-keto acid and the aminated form of the coenzyme, pyridoxamine 5'-phosphate which undergoes subsequent reconversion to

pyridoxal 5'-phosphate by reaction with an incoming alpha-keto acid to which the amino group is donated. By oscillating between the aldehyde and amino forms the coenzyme acts as a carrier of amino groups from amino acids to keto acids.

The aminotransferase reaction is represented schematically in Fig. 2. The pyridoxal 5'-phosphate enzyme complex is symbolized as H-CO-E and pyridoxamine 5'-phosphate - enzyme complex as $H_2N\text{-}CH_2\text{-}E$.

General kinetic properties

Kinetic analyses indicate that TAT has a lower affinity for tyrosine and 2-oxoglutarate than for pyridoxal 5'-phosphate (4, 5). Apparent Michaelis constants (K_m) for the substrates and coenzyme of TAT from rat, chicken and dog livers as well as the fungus, Trichoderma viride are given in Table 1.

Table 1: Km values for substrates of TAT from rat, chicken and dog livers and the fungus *Trichoderma viride*.

Organism		K_m Tyrosine (mol/l)	K_m 2-oxoglutarate (mol/l)	K_m pyridoxal 5'-phosphate (mol/l)	References
Rat	(i)	1.5×10^{-3}	6.5×10^{-4}	3.1×10^{-7}	7
	(ii)	1.4×10^{-3}	6.7×10^{-4}	1.7×10^{-8}	4
	(iii)	1.5×10^{-3}	1.1×10^{-3}	1.1×10^{-6}	29
Chicken		1.4×10^{-3}	6.6×10^{-4}	-	14
Dog		7.1×10^{-4}	1.6×10^{-4}	-	43
Trichoderma viride		5.0×10^{-4}	2.5×10^{-4}	-	11

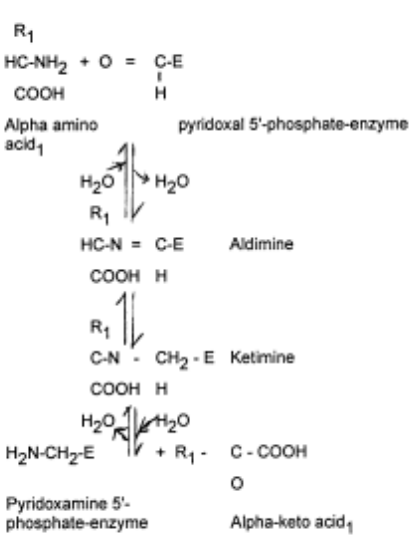
The reaction catalyzed by TAT has been analyzed with regard to product inhibition. Glutamate is alinear competitive inhibitor with respect to coenzyme or 2-oxoglutarate. pHPP is a linear non-competitive inhibitor with respect to tyrosine or pyridoxal 5'-phosphate but is a linear competitive inhibitor with respect to 2-oxoglutarate (5). These data indicate a ping-pong mechanism. The reaction between enzyme and substrates is first order with respect to tyrosine, pyridoxal 5'-phosphate and 2-oxoglutarate. Reduction of the concentration of 2-oxoglutarate to a rate-limiting value does not change the order with respect to tyrosine or pyridoxal 5'-phosphate. Similarly, reduction of the concentration of pyridoxal 5'-phosphate or of tyrosine or both to rate-limiting levels when the concentration of 2-oxoglutarate is varied does not affect the order of the reaction with respect to 2-oxoglutarate indicating that homotropic effects are not manifested (6).

Inhibitors of TAT activity

TAT is strongly inhibited by reagents which react with sulfhydryl groups such as p-chloromeruriphenyl sulfonate, o-iodosobenzoate or iodoacetate (7, 43). p-Chloromercuriphenyl sulfonate is about 100 times as effective as iodosobenzoate, which in turn is 100 times more effective than iodoacetate (7). Inhibition by these reagents and the partial reversal by appropriate mercaptans indicate that protein sulfhydryl is necessary for enzymatic activity.

Thyroxine, a hormone secreted bythe thyroid gland, uncompetitively inhibits TAT activity (7). At low enzyme levels considerable inhibition is obtained at concentrations of thyroxine which approach the physiological range. The inhibition cannot be reversed either by increasing the concentration of any of the enzyme substrates or by preincubation of the enzyme with its substrates before the addition of thyroxine. Thus, thyroxine appears to bind TAT at a site which does not involve substrate attachment.

1st stage



2nd stage

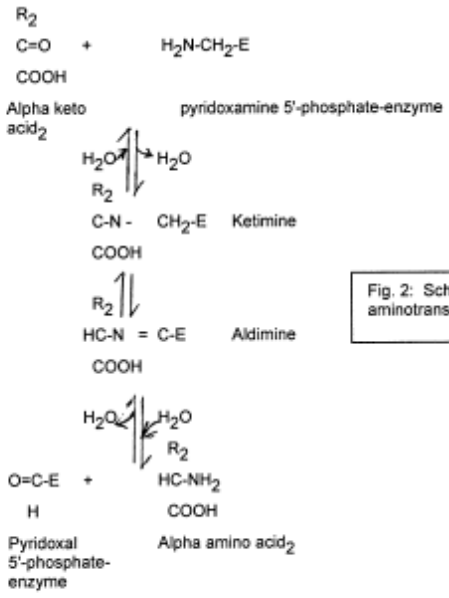


Fig. 2: Schematic representation of the aminotransferase catalyzed reaction

Various analogues of tyrosine, phenylalanine and tryptophan such as p-hydroxyphenylacetic acid, phnylactic acid and indole-3-butyric acid are competitive inhibitors of tyrosine transamination and are effective at 4 times the concentration of tyrosine (48). 3,4-Dihydroxyphenyl-alanine and its metabolic derivatives such as dopamine, norepinephrine and epinephrine as well as their methoxylated analogues are also effective competitive inhibitors of TAT (43). A rational explanation for inhibition by catechol compounds is provided by the fact that 3,4-dihydroxyphenylalanine is a substrate of TAT.

HORMONAL REGULATION OF TAT

Glucocorticoid-mediated induction of TAT

Glucocorticoids induce an increase in hepatic TAT activity (21, 49). The hormonal induction of enzyme activity is a consequence of an increase in the rate of synthesis of TAT (50 - 53). removal of glucocorticoids from fully induced liver cells results in a fall in TAT activity and a decline in its rate of synthesis. The decrease in activity is accompanied by a loss of antigenic activity, indicating that the enzyme is converted or degraded to a non-antigenic form (54). After addition of inducing steroid, there is a lag of about 30 min before an increase in enzyme activity is detected. The presence of the lag period suggests that the induction is sequential.

The increased rate of synthesis of TAT is due to a selective increase in the level of functional TAT mRNA in response to glucocorticoid administration. The first successful translation of polysomal mRNA coding for TAT into the enzyme was published independently in 1976 by Nickol *et al* (55) and by Roewekamp's group (56). Since then other reports have appeared in the literature describing translation of specific TAT mRNA into enzyme (21, 50, 57 - 62). Increased TAT activity after glucocorticoid stimulation is a direct result of increased rate of TAT mRNA formation, as anezyme induction is obtained only under conditions of increased transcription and does not take place after application of inhibitors of RNA synthesis (49, 63). Similar increases in the levels of translatable mRNA for proteins which undergo induction have been observed in a variety of tissues after various steroid hormone administration, suggesting hormonal control of specific gene transcription (64 - 66). Glucocorticoids do not affect the stability of TAT mRNA since its rate of turnover is constant during induction and withdrawal of the steroid hormone. There is a direct proportionality between TAT mRNA and the rate of synthesis of the enzyme. At no time during induction is there any TAT mRNA functionally viable but not actively engaged by the ribosomes in protein synthesis (55). Withdrawal of hydrocortisone does not affect the ability of available TAT mRNA to direct enzyme synthesis (56). This is in contrast with analysis of estrogen withdrawal in rooster liver, during which the induced vitellogenin mRNA is found to persist, although no longer actively engaged in vitellogenin synthesis (64).

Hepatic glucocorticoid receptors

Naturally occurring and synthetic glucocorticoids bind with high specificity to cytoplasmic receptor proteins within their target cells (61, 67 - 69). The steroid-receptor complex then transmigrates to the nucleus where it binds to chromatin. In rat liver cytosol, the G protein is the glucocorticoid receptor (70). Saturation of the G protein in vivo and its migration into the nucleus after cortisol administration correlates well with the extent of hormonal induction of TAT and tryptophan oxygenase, another glucocorticoid inducible enzyme (67).

Neither steroid, receptor protein nor newly formed steroid-receptor complex can bind DNA without first undergoing activation. In vitro studies indicate that at a temperature of 25o the receptor-glucocorticoid complex becomes transformed with the result that it rapidly interacts with DNA (71). The temperature-dependent stimulation of the steroid-receptor complex also occurs at 0^{o} though at a much slower rate; however, the presence of physiological concentration of calcium ions or isotonic concentrations of saline dramatically accelerate the activation process at this temperature (71). That physiological levels of Ca^{2+} are

capable of enhancing the transformation of the glucocorticoid-receptor complex suggests that Ca^{2+} may play a role not only in maintaining chromosomal structure but also in modulating hormone-receptor gene interaction and transcription. The steroid-receptor complex can also be activated by dilution or by subjecting it to gel filtration (72).

The exact chemical nature of the activation process is presently unknown. A conformational change appears to be necessary for nuclear binding and this involves exposure of positively charged regions on the surface of the receptor, resulting in an increse in the affinity of the steroid-receptor complex for DNA (71). Current thinking on the mechanism of steroid hormone action can be summarized as follows:

(i) Glucocorticoid enters the cytosol and forms a high affinity complex with the receptor.
(ii) Steroid-receptor complex undergoes one or more types of irreversible activation in the extranuclear compartment which may involve Ca^{2+} ions.
(iii) Activated steroid-receptor complex translocated into the nucleus and binds DNA-chromatin.

Anti inducers of TAT

A number of steroid have been classified as anti-inducers because at appropriate concentrations they inhibit the induction of TAT by glucocorticoids (73, 74). These inhibitory steroids include testosterone, 17 alpha-methyltestosterone, progesterone and 17 beta-estradiol. TAT is also inhibited by growth hormone (75). These hormones do not cause an increase in TAT activity even at relatively high concentrations. However, when the anti-inducers are used in combination with glucocorticoids, enzyme induction is inhibited (73). At high glucocorticoid concentrations and low concentrations of anti-inducer, the inhibition of TAT induction is completely reversed. Addition of an anti-inducer to pre-induced cells results in a prompt fall in enzyme activity to the level observed when glucocorticoid and the anti-inducer are added at the beginning of induction (74). Readjustment to the new rate of enzyme synthesis occurs almost immediately after addition of an anti-inducer. Inhibition of TAT induction by the anti-inducers is specific and not secondary to generalized inhibition of protein synthesis (76).

The effect of these steroids on the synthesis of TAT has been explained by proposing that these hormones interact with an allosteric receptor system (73). According to this model, uncomplexed receptors exist prediominantlyin an inactive conformation. Inducer binding promotes a shift toward an active conformation. Inducer binding promotes a shift towards an active conformation, while binding by anti-inducers does not cause an increase in the concentration of active receptors. Consistent with the model are the observations that uncomplexed receptors and receptors bound to anti-inducers are more thermolabite than inducer-bound receptors. Even if this model is valid, available evidence does not allow distinction between induced conformational changes or stabilization of pre-existing states.

Glucocorticoid-receptor complex accumulates in the cell nucleus while progesterone-bound receptors are unable to (76). The ability of the receptor to localize in the nucleus is a property of the active but not of the inactive conformational state. Although the anti-inducer-bound receptor has been termed inactive, it may still play a physiological role, for example, as a negative control element in the cytosol (74).

Glucagon - or cAMP-mediated induction of TAT

The effects of glucagon are mediated through its second messenger, cAMP. Replacement of glucagon by cAMP or active and logues of cAMP such as dibutyryl cAMP (bt_2 cAMP) leads to the induction of hepatic TAT essentially identical to that caused by the polypeptide hormone; in addition, glucagon and cAMP effects are not additive (77 - 79). The mechanism of action of cAMP on TAT induction is controversial. At least two independent sites of regulation which can be exerted at the levels of translation and transcription appear possible. In some liver

systems and hepatoma cells cAMP or bt_2 cAMPresults in an immediate increase in TAT activity without the lag period characteristic of steroid induction of the enzyme (80, 81). The presence of the lag time in glucocorticoid-regulated induction of TAT suggests that there may be intermediate steps between the binding of the steroid-receptor complex to chromatin and the induction of specific mRNAs (68). Removal of cAMP immediately inactivates the TAT synthesizing system and, moreover, the early induction of TAT synthesis by the cyclic nucleotide is not sensitive to actinomycin D (80, 81). These observations have led to the hypothesis that cAMP stimulates TAT through a translational process.

The translational model predicts that cAMP or bt_2cAMP should not alter the level of functional mRNA but direct measurements of the latter have shown that treatment with bt_2cAMP increases the concentration of mRNA coding for TAT (50, 59, 65, 66). The induction of enzyme activity by bt_2cAMP parallels the increase in mRNA levels and the magnitude of both increases are approximately proportional (50, 59). Glucocorticoids elicit a similar effect in the induction of TAT (56, 63). Actinomycin D or cordycepin blocks the induction of TAT synthesis by glucocorticoids in liver and hepatoma cell lines (63, 82). These agents also block the induction of TAT synthesis in liver systems that respond to bt_2-cAMP by increased formation of TAT mRNA (57, 83, 84) suggesting that continued transcription is required to maintain the induced state. In addition, a cAMP receptor protein has been reported which on binding to the cyclic nucleotide is translocated into the nucleus in a manner identical to the glucocorticoid-receptor complex (85).

It has been demonstrated that cAMP-dependent protein kinase mediates the effects of cAMP on the synthesis of TAT in Reuber H35 hepatoma cells (86). However, the site of action of the kinase on the induction of TAT in this system has not yet been established. TAT is phosphorylated in rat liver and in H35 hepatoma cells. Incorporation of (^{32}P) into the enzyme is rapid and is found exclusively as phosphoserine. Phosphorylation of TAT is not prevented to any significant extent by inhibition of protein synthesis and it is enhanced in the presence of glucocorticoids and insulin which do not activate the cAMP-dependent protein kinase. Thus, phosphorylation of the enzyme might not be involved in the regulation of its synthesis by cAMP.

Glucagon induction of amino acid transport; glucocorticoid permissive effect

Increased TAT synthesis in response to glucagon could result from the known activation of amino acid transport into liver cells by the polypeptide hormone; this would have important consequences on the rate of protein translation. Glucocorticoids enhance the induction of TAT by glucagon or bt_2cAMP (87). The ability of glucocorticoids to potentiate the metabolic effects mediated by glucagon has been termed the permissive effect. Most of these effects have been observed in the hormonal control of gluconeogenesis (88) where one aspect of this regulation is the induction of certain gluconeogenic enzymes including TAT.

Glucocorticoid permissive effect on glucagon induction of the transport of &-aminoisobutyric acid (AIB), a non-metabolizable analog of alanine has been demonstrated (89). Glucagon, bt_2cAMP or insulin can stimulate the transport of AIB. However, dexamethasone alone exerts no detectable effect but the synthetic glucocorticoid in combination with glucagon or bt_2cAMP results in an increase in AIB transport of more than 2-fold over that observed with glucagon or bt_2cAMP alone (89).

A model consistent with evidence from investigations and which reflects curent thinking about the nature of the permissive glucocorticoid effect on glucagon induction of AIB transport in cultured adult rat hepatocytes has been proposed by Pariza *et al* (89). The basic tenet of the model is the existence of a glucocorticoid-induced inactive amino acid transport system which is converted to an active transport system by a glucagon-mediated process. The model suggests that the effect of glucocorticoids may not be to increase cAMP levels in response to glucagon exposure but rather to induce the formation

of the inactive transport system. In support of this theory, theophylline, the cAMP phosphodiesterase inhibitor, is unable to mimic the action of bt_2cAMP and suggests that dexamethasone does not exert its permissive effect by inhibiting the degradation of cAMP (84).

The glucocorticoid permissive effect on the uptake of amino acids, sugars, purine and pyrimidine bases as well as nucleosides and metal ions in a variety of tissues is well established (89). Thus, the potentiation of the effect of bt_2cAMP by glucocorticoids is compatible with a permissive role for the steroid hormone where facilitated transport of amino acid, purine and pyrimidine bases results in increased synthesis of amino acid catabolizing enzymes such as TAT in liver cells (87).

The permissive effects of glucocorticoids on glucagon induction of amino acid transport are relatively short-lived in the absence of the steroid hormone (89). Thus, the maximum effect of glucagon in vivo occurs at or shortly following a peak of glucocorticoids whereas the minimum glucagon effect occurs when the steroid levels are low. Indeed, this may be the case in the report by Baril and Potter (90) where the oscillation of amino acid transport in vivo was abolished by adrenallectomy. The nature of the oscillation of glucocorticoids in vivo may regulate not only the potential level of glucagon induced activity but also the time required to reach the maximum induced level.

Insulin-mediated induction of TAT

TAT activity is elevated by insulin in a variety of liver systems (90, 91). Generally, insulin and glucagon have opposite effects in regulation of metabolism in the liver. Glucagon induces many key enzymes for amino acid catabolism and thus enhances gluconeogenesis and ureogenesis from amino acids, while insulin inhibits these activities increased by glucagon, but stimulates glycolysis and lipogenesis. Thus, the induction of TAT synthesis by insulin seems to be exceptional for this hormone.

The induction by insulin, unlike that stimulated by glucocorticoids occurs with a short lag of about 5 min and is insensitive to inhibition by actionomycin D, an indication that insulin acts on the translational step in TAT induction (92). Insulin stimulates a 30 - 40% increase in the rate of synthesis of both TAT and total cellular protein (93, 94). There is no selective increase in the rate of synthesis of TAT relative to total protein and Spencer *et al*., (93) have demonstrated that the larger increase in the cellular concentration of the enzyme is due to a selective dcrease in the rate of degradation of the enzyme by insulin. Thus, according to this hypothesis,the 2- to 3-fold increase in the amount of TAT observed with insulin is primarily as a result of a selective 2- to 3-fold increase in the half-life of the enzyme relative to total protein.

Other investigators have shown that insulin has a dual effect on TAT activity, i.e., an early effect and a late effect (95, 96). The early effect involves the stimulation of hepatic uptake of amino acids and polysome aggregation and hence general protein synthesis. This effect of insulin is secondary and short-lived. The late and primary effect of insulin on TAT activity is inhibitory, like its inhibition of induction of other amino acid degrading enzymes. This latter effect of insulin on TAT activity seems physiologically reasonable.

A generalized increase in total mRNAs after insulin administration to adrenalectomized rats with a resultant increase in total protein synthesis has been demonstrated (94). The mechanism of the increase in translatable hepatic mRNAs and perhaps in the larger increase in TAT mRNA is not known but Hill *et al*., (94) have suggested a change in mRNA structure rather than in other components of the translational machinery. Control of translation at the level of ribosomal initiation is well established and improved initiation has been implicated in the stimulation of protein synthesis by insulin in muscle and fat cells as well as in liver (97). A structural change in mRNAs elicited by insulin treatment and permitting improved initiation of translation could be responsible for the generalized effects on protein synthesis as well as for the apparent

induction of TAT synthesis and other specific proteins (94).

The molecular mechanism of insulin ation is still unclear. It would appear that there are at least three distinct but closely related routes through which insulin receptor might mediate the effects of the hormone in responsive cells. Current concepts of the mechanism of action of insulin have been reviewed (98) and are summarized as follows:

(i) Clusters of insulin-receptor complexes are formed on binding of the hormone to target cells. the insulin-receptor complexes are subsequently internalized into vesicles by an energy dependent process. The vesiculated complex of insulin receptor triggers specific intracellular processes after which they are degraded in lysosomes or recycled back to the plasma membrane.

(ii) The B-subunit of the insulin receptor undergoes auto-phosphorylation in the presence of insulin. This reaction activates the receptor which then phosphorylates specific protein substrates.

(iii) A specific guanine nucleotide regulatory protein is activated by interaction with the insulin-receptor complex. The active form of the guanine nucleotide protein then regulates the activities of specific plasma membrane-bound cAMP-dependent and independent protein kinases.

Thus, one or a combination of these routes is a mean by which the binding of insulin to its receptor at the cell surface might lead to the eliciting of the effects of the hormone on a wide variety of metabolic processes within the cell.

Superinduction of TAT

Superinduction is a term used to describe the apparent increase in specific mRNAs of some enzymes caused by the use of inhibitors of RNA synthesis such as actinomycin D and potnt inhibitors of protein biosynthesis like cycloheximide, emetine and puromycin (99, 100).

Superinduction has been demonstrated with TAT (57), tryptophan oxygenase (101) and a variety of other proteins (102). In the case of TAT two widely divergent explanations of superinduction of the enzyme have evolved. One concept is that large concentrations of actinomycin D inhibit the synthesis of a labile repressor of TAT mRNA activity (103). According to this notion superinduction should be characterized by TAT mRNA levels equal to or exceeding those seen with steroid treatment alone. The other view holds that actinomycin D merely merely stabilizes TAT against degradation and its mRNA activity should be at or near the uninuced level (104).

A direct assay of TAT mRNA activity under conditions in which TAT is superinduced shows that there is an increase in the activity of the enzyme and a specific decrease in its mRNA (57). Other reports show the same to be true for the superinduction of tryptophan oxygenase (101). In these two cases, at least, increased mRNA activity is precluded as a mechanism of this process and superinduction would appear to be related to stabilization of these enzymes against degradation as postulated by kenney (99).

However, the specific mRNAs for alpha 2U-globulin and interferon are increased in proportion to the superinduction of these proteins (102, 105). Thus, it would appear that there are several mechanisms responsible for superinduction just as there are for induction.

Concluding Remarks

The major difference between the transcriptional control of TAT induction by bt_2cAMP and glucocorticoid induction of the enzyme centres on the rapidity of the increase in TAT mRNA activity following treatment with the inducers. The lag in the appearance of increased levels of functional TAT mRNA during glucocorticoid induction is less than 30 min and is one of the most rapid primary responses to a steroid hormone yet demonstrated. The accumulation of TAT mRNA after stimulation with bt_2cAMP is even more rapid than that which occurs after glucocorticoid treatment. The rapidity of this response raises questions pertaining

to the site and mechanism of action of cAMP. However, the observation that RNA synthesis inhibitors block the induction of TAT synthesis by bt_2cAMP in some liver systems but fail to block the induction in other liver systems and hepatoma cells suggests that no single mechanism exists for a cAMP regulation of TAT.

REFERENCES

1. Knox, W.E. and LeMay-Knox, M. (1951). Oxidation in liver of L-tyrosine to acetoacetate through p-hydroxyphenylpyruvate and homogentisic acid. Biochem. J. 49, 686 - 693.
2. Dickson, A.J., Marston, F.A.O. and Pogson, C.I. (1981). Tyrosine aminotransferase as the rate-limiting step for tyrosine catabolism in isolated rat liver cells. FEBS Lett. 127, 28 - 32.
3. Tong, J.H., Stoochnoff, B.A., D'Ilorio, A. and Benoiton, N.L. (1973) Transamination of ring-substituted L-phenylalanines by an extract from rat liver mitochondria. Can. J. Biochem. 51, 407 - 411.
4. Hayashi, S., Granner, D.K. and Tomkins, G.M. (1967). Tyrosine aminotransferase: purification and characterization. J. Biol. Chem. 242, 3998 - 4006.
5. Rosenberg, J.S. and Litwack, G. (1970) Liver cytosol tyrosine aminotransferase: Product inhibition and interaction with substrates. J. Biol. Chem. 245, 5677 - 5684.
6. Litwack, G. and Cleland, W.W. (1968). Studies on th tyrosine aminotransferase mechanism. Biochemistry 7, 2072 - 2080.
7. Kenney, F.T. (1959). Properties of partially purified tyrosine -α-ketoglutarate trans-aminase from rat liver. J. Biol. Chem. 234, 2707 - 2712.
8. Holten, D., Wicks, W.D. and Kenny, F.T. (1967). Studies on the role of vitamin B6 derivatives in regulating tyrosine alpha-ketoglutarate transaminase activity in vitro and in vivo. J. Biol. Chem. 242, 1053 - 1059.
9. Ohisalo, J.J., Andersson, S.M. and Pispa, J.P. (1977). Partial purification and properties of frog liver tyrosine aminotransferase. Biochem. J. 163, 411 - 417.
10. Hargrove, J.L. and Granner, D.K. (1980). Purification of the native form of tyrosine aminotransferase from rat liver. Anal. Biochem. 104, 231 - 235.
11. Echetebu, C.D. (1982). Some properties of tyrosine aminotransferase from Trichoderma viride. J. gen. Microbiol. 128, 2735 - 2738.
12. Evans, P.J. (1982). Purification and electrophoretic analysis of rabbit cytosolic tyrosine aminotransferase. Enzyme 28, 82 - 87.
13. Scapin, S., Autuori, F., Baldini, P., Incerpi, S., Luly, P. and Sartori, C,. (1982) Tyrosine aminotransferase activity of frog (Rana esculenta) liver -II. Comparative aspects of intracellular distribution. Comp. Biochem. Physiol. 73B, 779 - 783.
14. Onoagbe, I.O. and Dickson, A.J. (1987). Tyrosine aminotransferase of chicken liver: purification and properties. Comp. Biochem. Physiol. 86B, 179 - 183.
15. Montemartini, M. (1993). Purification and partial structural and Kinetic characterization of tyrosine aminotransferase from epimastigotes of Trypanosoma cruzi. Biochem. J. 292, 901 - 906.
16. Valeriote, F.A., Auricchio, F., Tomkins, G.M. and Riley, D. (1969). Purification and properties of rat liver tyrosine aminotransferase. J. Biol. Chem. 244, 3618 - 3624.
17. Roewekamp, W., Sekeris, C.E. and Staerk, J. (1977). Purification and subunit structure of tyrosine aminotransferase from rat liver cytosol. FEBS Lett. 73, 225 - 228.
18. Lee, K.L., Roberson, L.E. and Kenney, F.T. (1979). Properties of tyrosine aminotransferase from rat liver. Anal. Biochem. 95, 188 - 193.
19. Lichtenstein, M.J., Potter, V.R. and Harper, A.E. (1974). Organ weight and tyrosine aminotransferase levels in the rat during adaptation to periodic fasting J. Nutr. 104, 1356 - 1364.
20. Nemeth, S. (1978). The effect of stress or glucose feeding on hepatic tyrosine aminotransferase activity and liver and plasma tyrosine level of intact and adrenalectomized rats. Horm. Metab. Res. 10, 144 - 148.
21. Granner, D.K. and Hargrove, J.L. (1983). regulation of the synthesis of tyrosine aminotransferase: the relationship to mRNA TAT. Mol. Cell. Biochem. 53/54, 113 - 128.
22. Goldberg, A.L. and St John, A.C. (1976). Intracellular protein degradation in mammalian and bacterial cells: part 2. Annu. Rev. Biochem. 45, 747 - 803.
23. Fellman, J.N., Vanbellighen, P.J., Jones, R.T. and Koler, R.D. (1969). Soluble and mitochondrial forms of tyrosine aminotransferase. Relaionship to human tyrosinemia. Biochemistry 8, 615 - 622.
24. Stellwagen, R.H. 91992). Involvement of sequences near both amino and carboxyl termini in the rapid intracellular degradation of TAT. J. Biol. Chem. 267, 23713 - 23721.

25. Belarbi, A., beck, G., bergmann, C. and Bollack, C. (1979). Intrinsic forms of soluble and mitochondrial tyrosine amino-transferase from rat tissues. FEBS Lett. 104, 59 - 65.
26. Miller, J.E. and Litwack, G. (1969). Studies on soluble and mitochondrial tyrosine aminotransferase: evidence for a physical change in the cytosol enzyme during induction. Biochem. Biophys. res. Commun. 36, 35 - 41.
27. Sapiro, V., Shear, L. and Litwack, G. (1974). translocation of inducible tyrosine aminotransferase to the mitochondrial fraction. J. Biol. Chem. 249, 2122 - 2129.
28. Miller, J.E. and Litwack, G. (1971). Purification, properties and identity of liver mitochondrial tyrosine aminotransferase. J. Biol. Chem. 246, 3234 - 3240.
29. Iwasaki, Y., Lamar, C., Danenberg, K. and Pitot, H.C. (1973). Studies on the induction and repression of enzymes in rat liver. Eur. J. Biochem. 34, 347 - 357.
30. Wurtman, R.J. and larin, F. (1968). A sensitive and specific isotopic assay for the estimation of tyrosine transaminase. Biochem. Pharmacol. 17, 817 - 818.
31. Igo, R.P., Mahoney, C.P. and Limbeck, G.A. (1968). Studies on tyrosine-alpha-ketoglutarate transaminase from bovine thyroid and liver disease. Biochim. Biophys. Acta 151, 88 - 98.
32. Kenney, F.T. (1962). Induction of tyrosine-α-ketoglutarate transaminase in rat liver. J. Biol. Chem. 237, 1605 - 1609.
33. Johnson, R.W., Roberson, L.E. and Kenney, F.T. (1973). Regulation of tyrosine aminotransferase in rat liver: x. Characterization and interconversion of the multiple enzyme forms. J. Biol. Chem. 248, 4521 - 4527.
34. Ohisalo, J.J. and Pispa, J.P. (1976). heterogeneity of hepatic tyrosine aminotransferase. Separation of the multiple forms from rat and frog liver by isoelectric focyussing and hydroxylapatite column chromatography and their partial characterization Acta Chem. Scand. (B) 30, 491 - 500.
35. Rodriguex, J.M. and Pitot, H.C. (1975). The interconversion of multiple forms of tyrosine aminotransferase. Biochem. Biophys. Res. Commun. 65, 510 - 518.
36. Gohda, E. and Pitot, H.C. (1980). purification and characterization of a factor catalyzing the conversion of the multiple forms of tyrosine aminotransferase from rat liver. J. Biol. Chem. 255, 7371 - 7379.
37. Hargrove, J.L. and Granner, D.K. 91981). Physical properties, limited proteolysis and acetylation of tyrosine aminotransferase from rat liver. J. Biol. Chem. 256, 8012 - 8017.
38. Smith, G.J., Pearce, P.H. and Oliver, I.T. (1976). A lysosomal factor that interconverts multiple forms of rat liver tyrosine aminotransferase. Life Sci. 19, 1763 - 1775.
39. Gohda, E. and Pitot, H.C. (1981). A new thiol proteinase from rat liver. J. Biol. Chem. 256, 2567 - 2572.
40. Hargove, J.L., Gohda, E., Pitot, H.C. and Granner, D.K. (1982). Cathepsin T (convertase) generates the multiple forms of tyrosine aminotransferase by limited proteolysis. Biochemistry 21, 283 - 289.
41. Donner, P., Wagner, H. and Kroger, H. (1978). Tyrosine aminotransferase from rat liver: A purification in three steps. Biochem. Biophys. Res. Commun. 80, 766 - 772.
42. Voigt, J. and Sekeris, C.E. (1978). A simplified procedure for the purification of rat liver tyrosine aminotransferase. Hoppe-Seylers Z Physiol. Chem. 359, 1363 - 1369.
43. Canellakis, Z.N. 7 Cohen, P.P. (1956). kinetic and substrate specificity study of tyrosine-α-ketoglutaric acid transaminase. J. Biol. Chem. 222, 63 - 71.
44. Fonnum, F., haavakldsen, R. and Tangen, O. (1964). transamination of aromatic amino acids in rat brain. J. Neurochem. 11, 109 - 118.
45. Sjoerdsma, A. and Lovenberg, W. (1970). Serotonin now: clinical implications of inhibiting its synthesis with para-chlorophenylalanine. Ann. Intern. Med. 73, 607 - 612.
46. Ryan, W.L. and Elliot, J.A. (1968). Fluorophenylalanine inhibition of tumours in mice on a phenylalanine deficient diet. Arch. Biochem. Biophys. 125, 797 - 803.
47. Bulos, B. and handler, P. (1965). Kinetics of beef heart glutamicalanine transaminase. J. Biol. Chem. 240, 3283 - 3294.
48. Jacoby, G.A. and La Du, B. (1964). Studies on the specificity of tyrosine α-ketoglutarate transaminase. J. Biol. chem. 239, 419 - 424.
49. Onoagbe, I. O. (1994). Effects o gluco-corticosteroids and insulin on tyrosine aminotransferase activity in isolated chick embryo hepatocytes and in intact embryos in ovo. Arch. Biochem. Biophys. 309, 58 - 61.
50. lynedjian, P.B., Auberger, P., Guigoz, Y. and LeCam, A. (1985). Pretranslational regulation of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase (GTP) synthesis by glucagon and dexamethasone in adult rat hepatocytes. Biochem. J. 225, 77 - 84.

51. Donohue, T.M. Jnr. and Tuma, D.J. (1988). The increase in hepatic tyrosine aminotransferase activity by ethanol administration involves acceleration of enzyme synthesis. Biochem. Biophys. Res. Commun. 155, 1471 - 1477.
52. Chesnokov, V.N. 91990). Hydrocortisone regulation and expression of tyrosine aminotransferase gene in various tissues of rat. Biomed. Biochim. Acta 49, 1177 - 1186.
53. Oshima, H. (1992). Modulation of glucocorticoid induction of tyrosine aminotransferase gene expression by variation in cell density. Endocrinology 130, 2106 - 2112.
54. Granner, D.K., Thompson, E.B. and Tomkins, G.M. (1970). Dexamethasone phosphate-induced synthesis of tyrosine aminotransferase in hepatoma tissue culture cells. J. Biol. Chem. 245, 1472 - 1478.
55. Nickol, J.M., Lee, K.L., Hollinger, T. and Kenney, F.T. (1976). Translation of messenger RNA specific for tyrosine aminotransferase in oocytes of Xenopus laevis. Biochem. Biophys. Res. Commun. 72, 687 - 693.
56. Roewekamp, W.G., Hofer, E. and Sekeris, C.E. (1976). Translation of mRNA from rat-liver polysomes into tyrosine aminotransferase and tryptophan oxygenase in a protein-synthesizing system from wheat germ. Eur. J. Biochem. 70, 259 - 268.
57. Diesterhaft, M., Noguchi, T. and Granner, D. (1980). regulation of rat-liver tyrosine aminotransferase mRNA by hydrocortisone and by N6, O2-dibutyryladenosine 3'5'-phosphate. Eur. J. Biochem. 108, 357 - 365.
58. Noguchi, T., Diesterhaft, M. and Granner, D. (1982). Evidence for a dual effect of dibutyryl cyclic AMP on the synthesis of tyrosine aminotransferase in rat liver. J. Biol. Chem. 257, 2386 - 2390.
59. Hashimoto, S., Schmid, W. and Shutz, G. (1984). Transcriptional activation of the rat liver tyrosine aminotransferase gene by cAMP. Proc. Natl. Acad. Sci. U.S.A. 81, 6637 - 6641.
60. Simons, S.S. Jr., mercier, L., Miller, N.R., Miller, P.A., Oshima, H., Sistare, F.D., Thompson, E.B., wasner, G. and Yen, P.M. (1989). Differential modulation of gene induction by glucocorticoids and antiglucocorticoids in rat hepatoma tissue culture cells. Cancer Res. 49, 22445 - 22525.
61. Yang, Y.L., Tan, J.X. 7 Xu, R.B. (1989). Down-regulation of glucocorticoid receptor andits relationship to the induction of rat liver tyrosine aminotransferase. J. Steroid Biochem. 32, 99 - 104.
62. Gadson, P. Jr., (1993). Differential expression of tyrosine amino-transferase by glucocorticoids and insulin. Biochim. Biophys. Acta 1173, 22 - 31.
63. Nickol, J.M., Lee, K.L. and Kenney, F.T. (1978). Changes in hepatic levels of tyrosine aminotransferase messenger RNA during induction by hydrocortisone. J. Biol. Chem. 253, 4009 - 4015.
64. Mullinix, K.P., Wetekam, W. 7 Deeley, R.G. (1976). Induction of vitellogenin synthesis by estrogen in avian liver: relationship between level of vitellogenin mRNA and vitellogenin synthesis. Proc. Natl. Acad. Sci. U.S.A. 73, 1442 - 1446.
65. Hod, Y., Morris, S.M. and hanson, R.W. (1984). Induction by cAMP of the mRNA encoding the cytosolic form of phosphoenol pyruvate carboxykinase (GTP) from the chicken. J. Biol. Chem. 259, 15603 - 15608.
66. Sasaki, K., cripe, T.P., Koch, S.R., Andreone, T.L., Petersen, D.D., Beale, E.G. and Granner, D.K. (1984). Multihormonal regulation of phosphoenolpyruvate carboxykinase gene transcription. J. Biol. Chem. 259, 15242 - 15251.
67. Beato, M., kalimi, M., Beato, W. and feigelson, P. (1974). interaction of glucocorticoids with rat liver nuclei: effect of adrenalectomy and cortisol administration. Endocrinology 94, 377 - 387.
68. King, R.J.B. (1984). Enlightenment and confusion over steroid hormone receptors. nature 312, 701 - 702.
69. Rigaud, G., Roux, J., Picklet, R. and Grange, T. (1991) In vivo footprinting of TAT gene: dynamic interplay between the glucocorticoid receptor and a liver-specific factor. Cell 67 (5), 977 - 986.
70. Koblinsky, M., beato, M., Kalimi, M. and Feigelson, P. (1972). Glucocorticoid-binding proteins of rat liver sytosol. ll. Physical characterization and properties of the binding proteins. J. Biol. Chem. 247, 7897 - 7904.
71. Kalimi, M., Beato, M. and Feigelson, P. (1973). Interaction of glucocorticoids with rat liver nuclei. l. Role of the cytosol proteins. Biochemistry 12, 3365 - 3371.
72. Goidl, J.A., Cake, M.H., Dolan, K.P., Parchman, L.G. and Litwack, G. (1977). Activation of the rat liver glucocorticoid-receptor complex. Biochemistry 16, 2125 - 2130.
73. Samuels, H.H. and Tomkins, G.M. (1970). Relation of steroid structure to enzyme induction in hepatoma tissue culture cells. J. Mol. Biol. 52, 57 - 74.

74. Rousseau, G.G.; Baxter, J.D. and Tomkins, G.M. (1972). Glucocorticoid receptors: relations between steroid binding and biological effects. J. Mol. Biol. 67, 99 - 115.
75. Shires, T.K. and Hargrove, J.L. (1982). growth hormone inhibition of tyrosine aminotransferase in primary cultures of rat liver cells. Biochim. Biophys. Acta 714, 170 - 172.
76. Baxter, J.D., Harris, A.W., Tomkins, G.M. and Coh, M. (1971). Glucocorticoid receptors in lymphoma cells in culture: relationship to glucocorticoid killing ability. Science 171, 189 - 191.
77. Gunn, T.M., Tilghmann, S.M., Hansen, R.W., Reshef, L. and Ballard, F.J. (1975). Effects of cyclic adenosine monophosphate, dexamethasone and insulin on phosphoenolpyruvate carboxykinase synthesis in Reuber H-35 hepatoma cells. Biochemistry 14, 2350 - 2357.
78. Shelly, L.L. and Yeoh, G.C. (1991). Effects of dexamethasone and cAMP on tyrosine aminotransferase expression in cultured fetal rat hepatocytes. Eur. J. Biochem. 199, 475 - 481.
79. Onoagbe, I.O. and Dickson,, A.J. (1992). effects of glucagon and an analogue of cAMP on tyrosine aminotransferase in isolated chick embryo hepatocytes Exp. Cell res. 201, 514 - 516.
80. Roper, M. and Wicks, W.D. (1978). Evidence for acceleration of the rate of elongation of tyrosine aminotransferase nascent chains by dibutyryl cyclic cAMP. Proc. Natl. Acad. Sci. U.S.A. 75, 140 - 144.
81. Snoek, G.T., Voorman, H.O. and Wijk, V.R. (1981). further evidence for translational regulation of tyrosine aminotransferase synthesis by dibutyryl cyclic AMP in Reuber H35 hepatoma cells. Biochim. Biophys. Acta 655, 107 - 112.
82. Wicks, W.D. and McKibbin, J.B. (1972). Evidence for translational regulation of specific enzyme synthesis by N6, O2 - dibutyryl cyclic AMP in hepatoma cell cultures. Biochem. Biophys. res. Commun. 48, 205 - 211.
83. Ernest, M.J. and Feigelson, P. (1978). Increase in hepatic tyrosine aminotransferase mRNA during enzyme induction by N6, O2-dibutyryl cAMP. J. Biol. Chem. 253, 319 - 322.
84. Evans, P.J. (1981). regulation of hepatic tyrosineaminotransferase. Biochim Biophys. Acta 677, 433 - 444.
85. Kallos, J. (1977). Photochemical attachment to cyclic AMP binding protein(s) to the nuclear genome. Nature 265, 705 - 710.
86. Boney, C., Fink, D., Schlichter, D., Carr, D. and Wicks, W.D. (1983). Direct evidence that the protein kinase catalytic subunit mediates the effects of cyclic AMP on tyrosine aminotransferase synthesis. J. Biol. Chem. 258, 4911 - 4918.
87. Gurr, J.A. and Potter, V.R. (1980). Independent induction of tyrosine aminotransferase activity by dexamethasone and glucagon in isolated rat liver parenchyma l cells in suspension and in monolayer culture in serum-free media. Exp. Cell Res. 126, 237 - 248.
88. Exton, J.H. (1972). Gluconeogenesis. Metabolism 21, 945 - 990.
89. Pariza, M.W., Kletzien, R.F., Butcher, R.F. and Potter, V.R. (1976). Inductions by hormones added singly, simultaneously or sequentially: what cultured hepatocytes can tell us about metabolic regulation in the whole animal. Adv. Enz. Regul. 14, 103 - 115.
90. Heaton, J.H., Schilling, E., Gelehrter, T.D., Rechler, M. and Spencer, C.J. (1980). Induction of tyrosine aminotransferase and amino acid transport in rat hepatoma cells by insulin and the insulin-like growth factor: multiplication-stimulating activity receptors. Biochim. Biophys. Acta 632, 192 - 203.
91. Iwamoto, Y., Wong, K.Y. and Goldfine, I.D. (1981). Insulin action in cultured HTC and H35 rat hepatoma cells: receptor binding and hormone sensitivity. Endocrinology 108, 44 - 51.
92. Gelehrter, T.D. (1976). Enzyme induction. New Engl. J. Med. 294, 589 - 595.
93. Spencer, C.J., heaton, J.H., Gelenhrter, T.D. and Garwin, J.L. (1978). Insulin selectively slows the degradation rate of tyrosine aminotransferase. J. Biol. Chem. 253, 7677 - 7682.
94. Hill, R.E., Lee, K.L. and Kenney, F.T. (1981). Effects of insulin on mRNA activities in rat liver. J. Biol. Chem. 256, 1510 - 1513.
95. Nakamura, T., Noda, C. and Ichihara, A. (1981). Two phase regulation of tyrosine aminotransferase activity by insulin in primary cultured hepatocytes of adult rats. Biochem. Biophys. Res. Commun. 99, 775 - 780.
96. Messina, J.L., Chatterjee, A.K., Strapko, H.T. and Weinstock, R.S. (1992). Short-and long-term effects of insulin on tyrosine aminotransferase gene expression. Arch. Biochem. Biophys. 298, 56 - 62.
97. Lyons, R.T., nordeen, S.K. and Young, D.A. (1980). Effects of fasting and insulin administration on polyribosome formation in rat epididymal fat cells. J. Biol. Chem. 255, 6330 - 6334.

98. Heyworth, C.M. and Houslay, M.D. (1983). Challenge of hepatocytes by glucagon triggers a rapid modulation of adenylate cyclase activity in isolated membranes. Biochem. J. 214, 93 - 98.
99. Kenney, F.T. (1967). Turnover of rat liver tyrosine transaminase : stabilization after inhibition of protein synthesis. Science 156, 525 - 528.
100. Ernest, M.J., delap, L. and feigelson, P.J. (1978). Induction of hepatic tyrosine aminotransferase mRNA by protein synthesis inhibitors. J. Biol. Chem. 253, 2895 - 2897.
101. Killewich, L., Schutz, G. and Feigelson, P. (1975). Functional level of rat liver tryptophan 2,3-dioxygenase mRNA during superinduction of enzyme with actinomycin D. Proc. Natl. Acad. Sci. U.S.A. 72, 4285 - 4287.
102. Chatterjee, B., Hopkins, J., Dutchat, D. and Roy, A.K. (1979). Superinduction of alpha 2U globulin by actinomycin D: evidence for drug mediated increase in alpha 2U messenger RNA. Proc. Natl. Acad. Sci. U.S.A. 76, 1833 - 1837.
103. Thompson, E.B.; Granner, D.K. and Tomkins, G.M. (1970). Superinduction of tyrosine aminotransferase by actinomycin D in rat hepatoma (HTC) cells. J. Mol. Biol. 54, 159 - 175.
104. Lee, K.L. and Kenney, F.T. (1971). Assessment of hormone action in cultured cells. Acta Endocrinol. 153, 109 - 125.