INTERFERENCE BY TRYPTOPHAN ANALOGUES OF AN IN VITRO ASSAY FOR LIVER TYROSINE TRANSAMINASE ACTIVITY

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Mammalian liver tyrosine transaminase (L-tyrosine: 2-oxoglutarate aminotransferase) has been widely used as a model to study glucocorticoid hormone specific gene modulation. Additionally, the manner in which this system responds is often employed to delineate factors controlling gene expression during: pathogenesis [1], shock [2], stress [3], circadian rhythms [4]; change in metabolic status after growth hormone [5,6], glucose [7], insulin [8], glucagon [7], irradiation or starvation [9], anaesthesia [10]; growth of hepatoma cells in culture [11], or isolated liver cells [12]; liver regeneration [13], foetal differentiation [14], and neoplastic transformation in rat hepatomas [15]. In view of this popularity, it becomes important to establish valid procedures to measure liver tyrosine transaminase (TT) levels.

The activity of hepatic TT is commonly measured by a colorimetric procedure where the development of a blue colour is proportional to the concentration of *p*-hydroxyphenylpyruvic acid (PHPP), in a manner analogous to the Fiske–Subbarow reaction for inorganic phosphate [16]. In the course of an investigation on adaptive enzyme changes in liver, selected tryptophan metabolites were found to interfere with this colorimetric method in vitro. The results are described here with a view to preventing inadvertent future errors.

The enzyme preparation was obtained by homogenizing mouse liver in 9 vol of ice cold 0.14 M KCl, 0.0025 M NaOH. All other chemicals were reagent grade and were used without further purification.

The standard reaction was carried out as follows: a buffer–substrate reagent for 30 flasks, prepared just before use by mixing from individual, refrigerated stock solutions, contained 75 ml of 0.25 M sucrose; 18 ml of 0.1 M α-ketoglutaric acid (dissolved in 0.25 M NaH_2PO_4, pH 7.4); 15 ml of 0.2 M phosphate buffer, pH 7.4; 6 ml of 2.8 mM pyridoxal phosphate; and 0.6 ml of 0.5 M diethyldithiocarbamate (DDC). To 3.8 ml of this solution were added 0.6 ml enzyme preparation in a 30 ml Erlenmeyer flask. After 3 min incubation at 38°C in a water bath, 1.2 ml of 0.01 M L-tyrosine were added. Incubation was carried on for an additional 10 min. The reaction was stopped by adding 0.6 ml 100% trichloroacetic acid (TCA). Mixtures were shaken immediately and filtered through Whatman No. 1 paper. A series of standard flasks contained 100–300 μg PHPP.

The colorimetric reaction was carried out as follows: to 2 ml of filtrates from blank, experimental and standard flasks was added 4 ml of the colour reagent (which consisted of: 0.75 ml of a 3% solution of ammonium heptamolybdata in 5N HCl; 1.25 ml of a 1% solution of KH_2PO_4 and 1.6 ml distilled water). The mixtures were shaken vigorously and then allowed to stand for 30 min at room temperature. The intensity of the blue colour was determined by absorbance at 850 nm.

Data in table 1 show that 5-hydroxytryptophan and 5-hydroxytryptamine (serotonin) were nearly equally effective in causing a 3-fold increase in TT activity in vitro. Tryptophan, phenylalanine (which also contains an aromatic residue) and its derivatives *para*-chlorophenylalanine and 3,4-hydroxyphenylalanine (DOPA), and the long chain aliphatic amino acid leucine – all appeared ineffective in altering TT activity in vitro. So, the kinetics of this in vitro 'activation' was measured with tryptophan and its analogues.

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Table 1
Specificity of indole ring analogues in altering the colorimetric assay for TT activity

Addition +	A_{850}
Water	0.235
Tryptophan	0.287
5-Hydroxytryptophan	0.689
Serotonin	0.728
Phenylalanine	0.203
3, 4-Hydroxyphenylalanine	0.251
p-Chlorophenylalanine	0.275
Leucine	0.293

+ All materials (1 mg) were added in a volume of 0.2 ml to the standard assay for TT as described in the text. Each value is the average of three separate determinations.

The intensity of TT reaction increased with progressively larger quantities (0–5 mg) of either 5-hydroxytryptophan or serotonin in vitro (fig. 1), although the recovery of exogenously added PHPP was already linear with 100–300 μg of the enzyme product. An effect of tryptophan was not noticeable under these conditions (data not shown). It was next determined whether these materials altered the catalytic activity of the enzyme template or the development of colour in the ammonium heptamolybdate – KH_2PO_4 reaction.

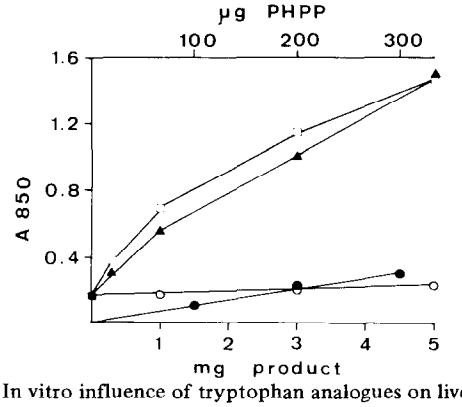

Fig. 1. In vitro influence of tryptophan analogues on liver tyrosine transaminase activity. Details of a standard assay are given in the text. The tryptophan (○), 5-hydroxytryptophan (□) or serotonin (▲) were dissolved in distilled water and the indicated amounts were added in a volume of 0.2 ml at the beginning of incubation. Flasks maintained as blands (■), or those containing the PHPP standard (●) received only distilled water. All values represent average of determinations on three separate enzyme preparations.

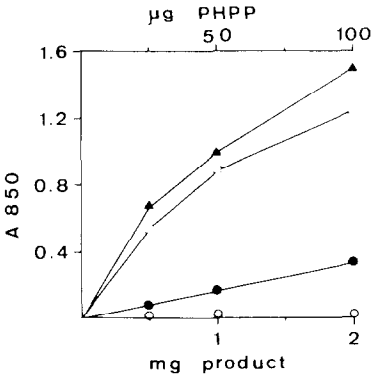

Fig. 2. Interference by tryptophan analogues of Briggs colour reaction for tyrosine transminase activity. Procedure for Briggs colour reaction has been described in the text. To 4 ml of the colour reagent were added increasing amounts of serotonin (□), tryptophan (○) or 5-hydroxytryptophan (▲), in a volume of 0.2 ml. A standard curve with PHPP was run at the same time (●). The samples were mixed thoroughly and the intensity of colour was measured 30 min later at 850 nm. All values represent average of three separate determinations.

As evident from fig. 2, addition of either 5-hydroxytryptophan or serotonin served to produce a product with strong absorption at 850 nm. However, quantitatively these compounds were not as specific as PHPP, and conceivably these materials could have an additive effect. Thus, reduction of phosphomolybdic acid to form a coloured complex is not specific to PHPP, although the latter may do so preferentially.

Liver TT activity may be measured by any one of a number of procedures. In the method of Diamondstone [17], the concentration of PHPP is assessed by its conversion to *p*-hydroxybenzaldehyde (PHBA), in presence of strong alkali, with an absorption maxima at 331 nm. This procedure suffers from the drawback that DDC (used to inhibit PHPP oxidase) absorbs strongly at 331 nm; the blanks are even higher (around 1.0), and very unstable, if TCA is used to precipitate protein. Moreover, the reaction requires high speed supernantant fractions which makes it impractical to process large numbers of individual samples. In the enolborate procedure [18], the increase in absorbance at 310 nm must be followed with time. Furthermore, tautomerase (for conversion of keto PHPP to the enol form) must be added separately under certain conditions. Although less sensitive than other assays, the chief advantages of the

modified Briggs colorimetric procedure (as employed here) are its overall simplicity and reliability as a fixed time assay for TT activity [19].

In an earlier report [20], serotonin and 5-hydroxytryptophan were shown to produce 6-fold induction in rat liver TT, as measured by the Briggs colorimetric procedure. The limited effectiveness of tryptophan in these in vivo experiments can most easily be explained by its hydroxylation and subsequent decarboxylation to products which would interfere with the phosphomolybdic reaction in vitro, as shown in this report. Since serotonin and 5-hydroxytryptophan were equally effective, and since tryptophan was inactive, it is clear that the ability to reduce the phosphomolybdic acid must reside in modification at the 5 position of the indole ring. The manner in which this is accomplished remains unknown. A comparative study with other techniques is required to assess whether these tryptophan analogues would indeed alter TT regulation in vivo.

In any event, it is obvious that the term 'induction' should be employed only when all evidence points to an increase in the amounts of protein moities. Thus, in future it becomes imperative to test the influence of a substance in vitro before attempting to arrive at a mechanism of the manner in which a change (either increase or decrease) may accompany administration of the substance in vivo.

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