ALTERNATE METABOLIC FATE OF 5-HYDROXYTRYPTOPHAN

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The compound 5-hydroxytryptophan (5-HT) is of interest because of its conversion to serotonin, and its excessive production by patients with carcinoid tumors. We present here evidence of an alternate metabolic fate of 5-HT which is catalyzed by enzymes which also metabolize tryptophan. The biological significance of this pathway is unknown but is under investigation.

The 2 indolylpyruvic acid (IPA) tautomerases catalyze equilibration of the keto and enol tautomers of IPA (Spencer & Knox, '59). During investigations of substrate specificity, the keto acid (5-hydroxyindolyl-pyruvic acid, 5-HIPA) generated from 5-HT was noted to be a suitable substrate for both tautomerases. Since there is evidence that 5-HIPA can be produced from 5-HT by transamination (Sandler et al, '60), this suggested a metabolic fate of 5-HT, distinct from its

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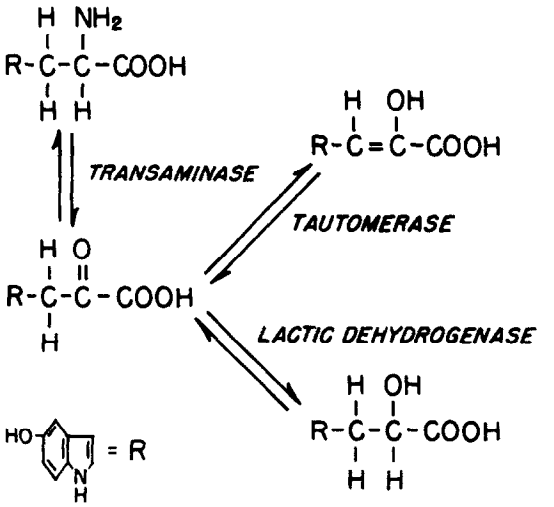

Figure 1. Alternate metabolic fate of 5-hydroxytryptophan.

decarboxylative conversion to serotonin. Evidence is presented that the hepatic enzyme involved in transamination of 5-HT with α-ketoglutarate is likely the same one that transaminates tryptophan. In addition, data indicating production of 5-hydroxyindolyllactic acid (5-HILA) from 5-HIPA are given. These metabolic interrelationships are shown in Figure 1.

EXPERIMENTAL

Assays were based on the enol borate-tautomerase method of Knox and Pitt ('57), which has been applied to the study of IPA metabolism (Spencer & Knox, '59, Spencer, '60). DL-5-HT was transaminated with α-ketoglutarate in borate by Civen's ('57) method (1 ml of supernatant from a 20% rat liver homogenate centrifuged at 105,000 x g for 30 minutes, 60 µgm pyridoxal phosphate, and 100 µmoles of DL-5-HT, in 0.57 M borate buffer, pH 8.1). The assay spectrophotometer cuvette received 20 µmoles of α-ketoglutarate to start the

reaction. Optical density (O.D.) changes were measured at 328 mμ, at which point 5-HT in borate does not absorb. After addition of the α-ketoglutarate, with the passage of time, there was a progressive increase in O.D. at 328 mμ (and spectral scans revealed a broad peak with maximum near this wavelength). When 5-HT was replaced in the assay of 50 μmoles of L-tryptophan, the same type of peak appeared. IPA, the product resulting from transamination of tryptophan, has been shown to absorb at 328 mμ (Bentley et al, '56).

Further evidence that the compound produced from 5-HT by transamination was 5-HIPA was provided as follows: 5-HT was oxidatively deaminated and the product shown to have the same spectrum as 5-HIPA produced by transamination. Cuvettes contained 4 mg L-amino acid oxidase, 1 mg catalase, borate buffer, and 0.5 μmoles 5-HT added at time zero to the assay cuvette. A spectral scan of the product resembled that obtained when 5-HT was transaminated. The reaction was usually performed with rat liver supernatant present to supply tautomerase and increase the rate of equilibration of the keto and enol tautomers. 5-HIPA is unstable and deteriorates after formation; 0.04 M disodium ethylenediamine tetraacetic acid slows the rate of degradation.

Transamination of 5-HT, measured by the O.D. at 328 mμ was zero order for at least 30 minutes. A typical reaction gave an increase of 0.15 O.D. units/10 minutes. When L-tryptophan was used in place of 5-HT, the increase was 0.17/10 minutes. Fractionation of rat liver supernatant by

solid ammonium sulfate (0-30%, 30-50%, 50% supernatant) and dialysis changed the specific activity of tryptophan-α-ketoglutarate transaminase, but the ratio of the activity on L-tryptophan and 5-HT (1.1:1) remained constant. This is consistent with the view that the same enzyme is involved in transaminating both tryptophan and 5-HT (as suggested by Sandler et al, '60, based on the adaptive increase of the enzyme).

That 5-HIPA was a suitable substrate for the IPA tautomerases was shown as follows: The L-amino acid oxidase system was used to convert 5-HT to 5-HIPA without tautomerase (rat liver supernatant). The increase in O.D. was slow. Upon addition of supernatant, the rate increased. Curves deviated slightly from those expected for a reversible first order reaction. Calculation of a reversible first order rate constant showed that addition of 1 ml of supernatant increased the rate of keto-enol tautomerization over 30 times. When N-ethylmaleimide was added to the supernatant, the activatable IPA tautomerase (that dependent upon glutathione for activity) was rendered inactive due to binding of its cofactor, but the catalysis of keto-enol tautomerization continued (however, at a slower rate). Hence both the activatable and basal (non-cofactor dependent) IPA tautomerases can utilize 5-HIPA as substrate. When DL-5-methyltryptophan was tested in the L-amino acid oxidase plus tautomerase system, it too was found to be a suitable substrate (hence 5-substitution does not affect the tautomerases). Partially

purified preparations of the IPA tautomerases also show the same effects.

Attempts to convert 5-HIPA to 5-HILA in the L-amino acid oxidase system by addition of lactic dehydrogenase and 0.74 μmoles DPNH (reduced diphosphopyridine nucleotide) directly were initially unsuccessful, as measured by a minimal decrease in O.D. at 340 mμ. The system also failed to convert IPA produced from tryptophan to indolyllactic acid. However, upon deproteinization in the cold with metaphosphoric acid and neutralization, the addition of lactic dehydrogenase and DPNH was followed by a decrease in O.D. at 340 mμ. Some component of the oxidative deamination system prevented the reaction, and the inhibiting agent was removed upon deproteinization. Synthetic IPA can also be shown to react with lactic dehydrogenase and DPNH (in borate buffer), by a decrease in O.D. at 340 mμ. This was likely a distinct process and not a nonspecific addition of an indole derivative to DPNH (Alivisatos et al, '60), since lactic dehydrogenase was required for the reaction.

In summary, 5-HT is converted to 5-HIPA by tryptophan-α-ketoglutarate transaminase. 5-HIPA is a suitable substrate for both of the IPA tautomerases. Evidence suggesting production of 5-HILA from 5-HIPA is cited.

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