Induction of Tryptophan Pyrrolase by α-Methyltryptophan and Its Metabolic Significance in Vivo*

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(Received for publication, April 11, 1963)

It is well known that the liver enzyme, tryptophan pyrrolase, increases rapidly and markedly in effective concentration when tryptophan is administered to animals (1). Earlier studies in this laboratory (2) demonstrated that α-methyltryptophan also induces an increase in this enzyme. Given and Knox then showed (3) that α-methyltryptophan, like tryptophan itself, is effective in this respect in adrenalectomized animals, thus distinguishing these two compounds from many others whose effects upon pyrrolase are mediated solely through hormonal mechanisms. Recently, Sankoff and Sourkes (4) reported that a single injection of α-methyltryptophan provokes (a) a loss of weight not explained by anorexia and (b) a long lasting increase in the hepatic pyrrolase level in the rat. They postulated that the weight loss is attributable to excessive oxidation of dietary and endogenous tryptophan, resulting in the diminished availability of this amino acid—in effect, an acute amino acid imbalance. Thus, it seemed pertinent to determine whether increased pyrrolase levels actually do play a major role in the physiological disposition of tryptophan.

EXPERIMENTAL PROCEDURE

DL-Tryptophan-β-C14 (dl-α-amino-β-(3-indole)propionic acid-β-C14; 3.7 mc per mmole) was purchased from New England Nuclear Corporation, and DL-tryptophan-α-C14 (dl-α-amino-β-(3-indole)propionic acid-α-C14; 3.4 mc per mmole) from Chem-Trac Corporation, Cambridge, Massachusetts. dl-α-Methyltryptophan was a gift from Merck Sharp and Dohme Research Laboratories. L-tryptophan was purchased from California Nuclear Corporation, and n-n-tryptophan-cr-C14 (m-a-amino-p-(3-indole)propionic acid-cr-C14; 3.4 mc per mmole) from Chem-Trac Corporation for Biochemical Research. A total of 40 rats, weighing 105 to 170 g, were used in this study. Each rat received an intraperitoneal injection of 0.2 mg of tryptophan-C14; some animals also received, at the same time or at various time intervals before the labeled tryptophan, 1 mmole of either L-tryptophan or DL-α-methyltryptophan as a suspension per kg of body weight by the same route. Controls received 0.9% NaCl solution.

Immediately after the injection of the radioactive compound, the animals were placed, without food or water, in individual metabolism cages allowing for the collection of CO2, feces, and urine. Usually six rats were used in a given experiment. Dry, CO2-free air was aspirated in series through the cage, through a trap containing 50 ml of 11.7 M KOH, and finally through a second trap containing 15 ml of KOH. Aliquots of the first KOH solution were taken at suitable time intervals; the entrapped CO2 was precipitated as BaCO3, and this was plated on tared planchets. Samples were counted with an ultrathin window gas flow counter coupled to an Atomic Instruments scaler. Counting was continued to a total of 1000 to 2000 counts (s.d. = ±3%) and was corrected for self-absorption. Corrections were made for any CO2 escaping to the second trap, and results are expressed as a percentage of the administered C14 appearing in the respiratory CO2.

RESULTS

Effect of l-Tryptophan on Tryptophan Pyrrolase in Vivo—The effect of a tryptophan load on the metabolism of tryptophan-β-C14 can be seen in Fig. 1. Recovery of administered C14 from control animals was about 9% in the first 10½ hours, but the recovery from rats that had received a load of unlabeled tryptophan 1 hour beforehand was 4 to 5 times greater. This shows that the induced increase in tryptophan pyrrolase effectively increases the rate of oxidation of tryptophan in vivo. With increasing times of pretreatment with the unlabeled tryptophan load (Fig. 2), the recovery of administered C14 approaches control values and with a pretreatment time of 24 hours, the recovery was the same as with controls. These findings agree with results previously obtained by assay in vitro of enzymatic levels (4, 5).

In Fig. 2, it can be seen that when both the labeled and unlabeled tryptophan are administered together, the recovery of C14 in the respiratory CO2 is greatest. In this case, one would expect dilution of the isotope by some of the 30 mg of cold material injected simultaneously with 0.2 mcg of radioactive tryptophan. The administration of the tryptophan load as a suspension precluded calculation of the dilution. However, the increased rate of oxidation presumably surpasses any dilution effect.

Similar results were obtained with dl-tryptophan-α-C14. When lower doses of the tryptophan load were used, recovery of C402 in the respiratory gases was considerably lower (Table III). The log dose-response relation is not linear.

Effect of α-Methyltryptophan—Previous studies (2, 4) have shown that injected α-methyltryptophan is more effective than tryptophan in increasing the activity of tryptophan pyrrolase.
in liver preparations as measured in vivo. However, from Fig. 1 it can be seen that the effect on tryptophan oxidation in vivo, as measured by C\textsuperscript{14} recovery, is not as marked. Experiments encompassing a wide range of pretreatment times are illustrated in Fig. 2. It is clear that although the amplitude is not as great, the effect is longer lasting than when a tryptophan load is given. Thus, α-methyltryptophan reaches its peak effect when it is injected 14 to 24 hours before the tryptophan load is given. However, even after 7 days the oxidation of tryptophan-\textsuperscript{14} is still 2 to 3 times above control values. Experiments with tryptophan-\textsuperscript{14} gave essentially the same results.

Effect of L-Kynurenine—In two experiments, L-kynurenine (1 mmole per kg) was injected with tryptophan-\textsuperscript{14}. There was no significant deviation in the percentage of C\textsuperscript{14} recovered (Table I). As an increased rate of oxidation of tryptophan would lead to large increases in the amount of kynurenine formed, this result indicates that the excess of metabolites coming from a tryptophan load would not be expected to affect the yield of respiratory CO\textsubscript{2}.

Urinary C\textsuperscript{14} Metabolites—Total urinary C\textsuperscript{14} metabolites were also determined. In Table II it can be seen that there is a corresponding increase in the radioactive metabolites from the urines of rats receiving L-tryptophan and α-\textsuperscript{14} methytryptophan loads. There is a good correlation between increased C\textsuperscript{14} in the respiratory gases and increased C\textsuperscript{14} metabolites in the urine.

### DISCUSSION

Sourkes and Townsend (2) previously demonstrated that α-methyltryptophan has several actions in relation to tryptophan pyrrolase: (a) it causes an induction of the enzyme; (b) it stabilizes tryptophan pyrrolase against spontaneous inactivation (6); (c) it can be shown to inhibit the enzyme; (d) it is not a substrate for the enzyme. The present results should be evaluated in this light. For example, after administration of α-methyl-
tryptophan, its concentration in body fluids, liver cells, or both could remain high for a longer period of time than tryptophan does when that amino acid is given. This would account for the observed increase in pyrrolase activity over a prolonged period. The fact that the maximal effect of DL-α-methyltryptophan known to lead to prolonged increase in the enzyme activity in liver also results in a prolonged and elevated increase in the rate of oxidation of tryptophan. These findings indicate that tryptophan pyrrolase plays a major physiological role in the control of catabolism of tryptophan in the rat.

**SUMMARY**

The effect of L-tryptophan and DL-α-methyltryptophan loading (1 mmole per kg) on the catabolism of DL-tryptophan-α-C\(^4\)\(^2\) and β-C\(^4\)\(^2\) has been studied to determine whether those unlabeled amino acids, which are known to induce tryptophan pyrrolase, enhance the breakdown of tryptophan in vivo in the rat. When L-tryptophan was injected concurrently with DL-tryptophan-β-C\(^4\)\(^2\), 39% of the C\(^4\)\(^2\) was recovered in the respiratory CO\(_2\) in 6 hours. When the tryptophan load was injected before the labeled tryptophan, CO\(_2\) production fell off, attaining control values (6.8% in 6 hours) with a 14-hour interval. DL-α-Methyltryptophan exerted its peak effect when injected 14 to 24 hours before the radioactive tryptophan, yielding 23% of the label in 6 hours. With longer injection intervals, there was a gradual decline in the percentage of C\(^4\)\(^2\) recovered, but the values remained 2 to 3 times control values even with 7 days of pretreatment.

**Acknowledgment**—The authors wish to thank Dr. Stephen Sved for much helpful advice during the course of this work.

**REFERENCES**


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**Table III**

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<tr>
<th>Labeled tryptophan administered</th>
<th>Reference</th>
<th>Dose</th>
<th>Recovered in respiratory CO(_2)</th>
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<td>3α,7α,7-C(^4)(^2)</td>
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</table>

* Estimated range of dose.
† Administered in two equal doses 6 hours apart.
‡ Present investigation.

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*tryptophan, its concentration in body fluids, liver cells, or both could remain high for a longer period of time than tryptophan does when that amino acid is given. This would account for the observed increase in pyrrolase activity over a prolonged period.*

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