Induction of Hepatic Tyrosine Aminotransferase by Indole Amines*

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SUMMARY

L-Tryptophan, L-5-hydroxytryptophan, serotonin, and other indole amines increase tyrosine aminotransferase activity in mice liver. L-5-Hydroxytryptophan was as effective as serotonin, whereas L-tryptophan was far less effective at low doses. If inhibitors of aromatic L-amino acid decarboxylase were injected prior to the administration of indole compounds, the increase of tyrosine aminotransferase activity by 5-hydroxytryptophan or tryptophan was completely blocked, whereas the increase by serotonin was not reduced. On the other hand, a monoamine oxidase inhibitor enhanced the increase of tyrosine aminotransferase activity by serotonin or 5-hydroxytryptophan. These results indicate that indole amines are the active agents in the increase of enzyme level. Among various indole amines, serotonin, 5-methoxytryptamine, and tryptamine were effective in increasing the enzyme activity. Although the increase in the enzyme activity was partially mediated by adrenal hormones, these indole amines could increase tyrosine aminotransferase activity in adrenalectomized mice. Cycloheximide blocked the increase of tyrosine aminotransferase activity by serotonin, indicating that the increase is due to induction of new enzyme. The results suggest a possible role of indole amines in the regulation of metabolism.

Hepatic tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) is inducible by various agents: glucocorticoids (1-3), glucagon (4), insulin (4), pyridoxine (5), epinephrine (6), and cyclic 3', 5'-adenosine monophosphate (7). Kenney and Flora (8) showed that L-tryptophan increased tyrosine aminotransferase activity in intact rats, but not in adrenalectomized rats. In subsequent studies, Rosen and Milholland (9) demonstrated that L-tryptophan, L-5-hydroxytryptophan, and serotonin could increase hepatic tyrosine aminotransferase activity in intact rats, but not in adrenalectomized rats. In intact rats, the increase of enzyme activity was as inducing agents and that the increased enzyme activity involves synthesis of new enzyme protein.

EXPERIMENTAL PROCEDURE

Indole compounds were purchased from Calbiochem or Regis Chemical Company, Chicago, Illinois, MK-486 (L-α-hydrazoneethyl-β-hydroxyphenylalanine) was kindly supplied from Merck Sharp, and Dohme Research Laboratories, West Point, Pennsylvania. Ro 4-4609 (N-(DL-seryl)-N'-2,3,4-trihydroxybenzyl)hydrazine) was kindly donated from Hoffman-La Roche Company, Nutley, New Jersey. Pargyline hydrochloride was kindly supplied from Abbott Laboratories, North Chicago, Illinois.

All compounds were dissolved in isotonic NaCl and neutralized to pH 6.5 to 7.5, and administered by intraperitoneal injection to mice. When indicated, the compounds were injected as suspensions at neutral pH.

Swiss-Webster male mice, weighing 20 to 22 g, were supplied from Simonsen Laboratories, Gilroy, California. Twenty mice were housed in one cage, and kept under controlled light (light on from 7:00 a.m. to 7:00 p.m.). All mice were deprived of food from 9:00 a.m. Water was available ad libitum. Each group consisted of 5 to 10 mice, and the experiments were repeated two or three times.

Adrenalectomized mice were bilaterally adrenalectomized under a Luxo lamp and the animals were maintained on a normal diet and 0.9% NaCl solution drinking water. Adrenalectomized mice were used for experiments 6 or 7 days after adrenalectomy. At the time of killing, it was confirmed that no residual adrenal remained.

Since tyrosine aminotransferase activity shows circadian variations, all mice were killed between 1:00 p.m. and 3:00 p.m., and the livers were quickly removed and frozen on Dry Ice. Tyrosine aminotransferase activity was measured within 24 hours after killing. Liver was homogenized in 5 volumes of 0.14 M KCl containing 0.02 M potassium phosphate buffer (pH 7.0), and centrifuged at 35,000 X g for 20 min. Tyrosine aminotransferase activity was assayed by the method of Diamondstone (10) using the supernatant.

For the measurement of serotonin, brain or liver was homogenized in 5 volumes of 4% perchloric acid containing 0.2% EDTA,
RESULTS

Increase of Tyrosine Aminotransferase by Indole Compounds—Serotonin or L-5-hydroxytryptophan was injected intraperitoneally into mice, and the mice were killed 2, 4, 6, and 9 hours after the injection. As shown in Fig. 1, tyrosine aminotransferase activity increased 3-fold 2 hours after injection of serotonin, reaching a maximum value at 4 hours. At the maximum, tyrosine aminotransferase activity was 4-fold higher than the basal level. Nine hours after the injection, tyrosine aminotransferase activity returned to the initial level. An identical pattern was obtained with L-5-hydroxytryptophan. In the following experiments, therefore, tyrosine aminotransferase activity was measured 4 hours after injection of indole compounds.

The dose response of tyrosine aminotransferase to serotonin, L-5-hydroxytryptophan, and L-tryptophan is shown in Fig. 2. At a dose of 0.05 mmole of serotonin, tyrosine aminotransferase activity increased 2-fold, with a 4-fold increase at a dose of 0.5 mmole. L-5-Hydroxytryptophan was less effective at doses lower than 0.5 mmole. At a dose of 0.5 or 1.0 mmole of L-5-hydroxytryptophan, tyrosine aminotransferase activity increased 4-fold to the same level as with serotonin. On the other hand, L-tryptophan was far less effective than serotonin or L-5-hydroxytryptophan. At a dose of 0.5 mmole or less, no significant increase was observed. At a dose of 1.0 mmole, tyrosine aminotransferase activity increased 2-fold, which was statistically significant. At a dose of 2 mmoles, tyrosine aminotransferase activity increased 4-fold as shown in Fig. 3.

Various indole compounds were injected at a dose of 0.5 mmole per kg body weight, and tyrosine aminotransferase activity was measured. As shown in Table I, serotonin creatinine sulfate, serotonin hydrogen oxalate, and free serotonin resulted in the same amount of increase of tyrosine aminotransferase activity, indicating that the increase was due to serotonin moiety. 5-Methoxytryptamine and tryptamine increased tyrosine amino-

![Image](https://example.com/image1)

**Fig. 1.** Time course of increase of tyrosine aminotransferase. Serotonin creatinine sulfate (5-HT) or L-5-hydroxytryptophan (L-5-HTP) was injected intraperitoneally at a dose of 0.5 mmole per kg of body weight, and the mice were killed at various time intervals as indicated. Vertical bars indicate standard errors of the mean.

![Image](https://example.com/image2)

**Fig. 2.** Dose responses of tyrosine aminotransferase activity. Serotonin creatinine sulfate (5-HT), L-5-hydroxytryptophan (L-5-HTP), or L-tryptophan (L-TP) was injected intraperitoneally at the doses indicated. Four hours after injection of the compounds, the mice were killed and tyrosine aminotransferase activity was measured. Vertical bars indicate standard errors of the mean.

![Image](https://example.com/image3)

**Fig. 3.** Effect of MK-486 on increases of tyrosine aminotransferase activity. MK-486 (0.2 or 0.3 mmole per kg of body weight) or 0.9% NaCl solution (Saline) was injected intraperitoneally 30 min prior to intraperitoneal injection of serotonin creatinine sulfate (5-HT), L-5-hydroxytryptophan (L-5-HTP), or L-tryptophan (L-TP), at the doses indicated. The mice were killed 4 hours after the injection of indole compounds, and tyrosine aminotransferase activity was measured. Vertical bars indicate standard errors of the mean.
Compounds injected

- NaCl
- Serotonin creatinine sulfate
- Serotonin hydrogen oxalate
- Serotonin (free)
- 5-Methoxytryptamine
- Tryptamine
- N-Acetylserotonin
- N-Methyltryptamine
- Melatonin
- 6-Hydroxymelatonin
- 5-Hydroxytryptophol
- 5-Hydroxy-3-indole acetic acid

Effect of aromatic L-amino acid decarboxylase inhibitors—Although indole amino acids or amines could increase tyrosine aminotransferase activity, the question remains whether these compounds by themselves or some metabolites of these compounds are responsible for the increase of the enzyme activity. One of the main metabolic pathways of L-5-hydroxytryptophan and L-tryptophan is decarboxylation of these amino acids to the corresponding amines. The effect of inhibitors of aromatic L-amino acid decarboxylase, therefore, was investigated. Among various inhibitors, MK-486 and Ro 4-4602 were employed in this study, because these two inhibitors are the most potent and long lasting inhibitors, and because the pattern of inhibition of the two drugs is different (12, 13). MK-486 inhibits aromatic L-amino acid decarboxylase in the peripheral tissues, but not in the brain, whereas Ro 4-4602 inhibits aromatic L-amino acid decarboxylase both in the peripheral tissues and in the brain. As shown in Fig. 3, MK-486 did not show a significant effect on the basal level of tyrosine aminotransferase activity. It did not block the increase of the enzyme activity by serotonin at any doses. On the other hand, pretreatment with MK-486 blocked the increase of tyrosine aminotransferase activity by L-5-hydroxytryptophan or L-tryptophan. At a dose of 0.2 mmole of MK-486, the blockage was not complete, whereas 0.3 mmole of MK-486 resulted in complete blockage of the increase of the enzyme activity.

Ro 4-4602 showed the same tendency. As shown in Fig. 4,

![Graph](image_url)

**Table I**

| Compounds Injected                  | Tyrosine aminotransferase μmoles/g/hr |
|-------------------------------------|---------------------------------------|
| NaCl                                | 122 ± 13                               |
| Serotonin creatinine sulfate        | 484 ± 53*                              |
| Serotonin hydrogen oxalate          | 509 ± 29*                              |
| Serotonin (free)                    | 477 ± 44*                              |
| 5-Methoxytryptamine                 | 411 ± 26*                              |
| Tryptamine                          | 313 ± 27*                              |
| N-Acetylserotonin                   | 173 ± 12*                              |
| N-Methyltryptamine                  | 171 ± 25                               |
| Melatonin                           | 160 ± 18                               |
| 6-Hydroxymelatonin                  | 147 ± 19                               |
| 5-Hydroxytryptophol                 | 149 ± 8                                |
| 5-Hydroxy-3-indole acetic acid      | 165 ± 23                               |

* Differs from NaCl group at p < 0.01.
* Differs from NaCl group at p < 0.05.

![Graph](image_url)

**Fig. 4.** Effect of Ro 4-4602 on increases of tyrosine aminotransferase activity. Ro 4-4602 (0.5 mmole per kg of body weight) was injected intraperitoneally 30 min prior to intraperitoneal injection of serotonin creatinine sulfate or L-5-hydroxytryptophan at the dose indicated. Four hours after injection of serotonin or L-5-hydroxytryptophan, the mice were killed, and tyrosine aminotransferase activity was measured. Vertical bars indicate standard errors of the mean.

![Graph](image_url)

**Fig. 5.** Effect of MK-486 or Ro 4-4602 on decarboxylation of L-5-hydroxytryptophan. MK-486 (0.3 mmole per kg) or Ro 4-4602 (0.5 mmole per kg) was injected intraperitoneally 30 min prior to intraperitoneal injection of L-5-hydroxytryptophan (0.5 mmole per kg). After various time intervals, the mice were killed and serotonin levels in the liver or in the brain were measured. Vertical bars indicate standard errors of the mean.
Ro 4-4602 has no effect on the increase of the enzyme activity by serotonin, whereas it completely blocked the increase of the enzyme activity by L-5-hydroxytryptophan.

The in vivo effect of these inhibitors on decarboxylation of 5-hydroxytryptophan was shown in Fig. 5. After the injection of 5-hydroxytryptophan, a large amount of serotonin accumulated in the liver, decreasing to the basal level 4 hours after the injection. On the other hand, MK-486 or Ro 4-4602 completely blocked decarboxylation of 5-hydroxytryptophan in the liver. In the brain, MK-486 slightly increased the accumulation of serotonin. Ro 4-4602 showed a different effect on decarboxylation of 5-hydroxytryptophan in the brain. Fifteen minutes after the injection of 5-hydroxytryptophan, serotonin levels in the brain did not increase, with a moderate increase 1 and 4 hours after the injection of 5-hydroxytryptophan.

The results shown above are taken to show that decarboxylation of L-5-hydroxytryptophan or L-tryptophan is essentially required for the increase of tyrosine aminotransferase activity by these amino acids.

Effect of Monoamine Oxidase Inhibitor on Increase of Tyrosine Aminotransferase—The main metabolic pathway of indole amines is oxidation of amines to acids by monoamine oxidase (EC 1.4.3.4). An inhibitor of monoamine oxidase, pargyline, was injected prior to injection of indole compounds. As shown in Fig. 6, pargyline has no effect on the basal level of tyrosine aminotransferase activity. Pargyline, however, greatly enhanced the increase of the enzyme activity by serotonin or L-5-hydroxytryptophan, indicating that serotonin, but not its metabolites derived via monoamine oxidase is responsible for the increase of the enzyme activity. The level of the enzyme activity after the injection of L-tryptophan alone or L-tryptophan plus pargyline was not significantly different from the level in the control groups, although there was a tendency for an elevation after pretreatment with monoamine oxidase inhibitor.

Increase of Tyrosine Aminotransferase Activity in Adrenalectomized Mice—Serotonin as well as tryptamine increased plasma corticosterone level 30 min after injection. NaCl (0.9%) injections also increased plasma corticosterone level by 50%. Adrenalectomized mice, therefore, were used in the following experiments. Indole amino acids and amines were tested for the increase of tyrosine aminotransferase activity and the results are expressed as mean ± standard error of the mean.

### Table II

| Compounds injected | Dose | Tyrosine aminotransferase |
|--------------------|------|---------------------------|
|                    | mmol/kg | µmol/g/hr |
| NaCl               |        |            |
| L-Tryptophan       | 1.0    | 182 ± 13³|
| Serotonin creatinine sulfate | 0.2 | 204 ± 20¹ |
|                    | 0.5    | 206 ± 11¹ |
| L-5-Hydroxytryptophan | 1.0  | 227 ± 24¹ |
| 5-Methoxytryptamine | 1.0    | 273 ± 23¹ |
| Tryptamine         | 1.0    | 225 ± 21¹ |

[¹, ³] Differs from NaCl group at p < 0.05.
[²] Differs from NaCl group at p < 0.01.
was injected intraperitoneally 30 min prior to intraperitoneal injection of serotonin creatinine sulfate (0.2 mole per kg). Four hours after the injection of serotonin, the mice were killed and tyrosine aminotransferase activity was measured. Results are expressed as mean ± standard error of the mean.

| Treatment                      | Tyrosine aminotransferase activity (μmoles/g/hr) |
|--------------------------------|--------------------------------------------------|
| NaCl                           | 103 ± 7                                          |
| Serotonin                      | 221 ± 24*                                        |
| Cycloheximide                  | 120 ± 9                                          |
| Cycloheximide + serotonin      | 133 ± 14                                         |
| Actinomycin D                  | 119 ± 12                                         |
| Actinomycin D + serotonin      | 192 ± 9*                                         |

* Differs from NaCl group at p < 0.01.

The results reported in this paper indicate that serotonin induces synthesis of new enzyme, although the possibility that indole amines increased tyrosine aminotransferase activity by decreasing the degradation of the enzyme has not been excluded. Actinomycin D did not block the increase of tyrosine aminotransferase activity. This result is inconsistent with the reports by other investigators (15, 16) that the induction of tyrosine aminotransferase by L-tryptophan was completely blocked by actinomycin D. It might be possible that actinomycin D blocks the induction by L-tryptophan by other unknown processes.

Some of the effect of indole compounds is mediated by adrenal hormones, because these indole compounds elevate plasma corticosterone level, and because adrenalectomy reduces the amount of increase of tyrosine aminotransferase activity by indole compounds. These indole compounds, however, could increase tyrosine aminotransferase activity in adrenalectomized mice, suggesting that some other mechanism than adrenal hormones is also involved in the induction. Several possibilities are conceivable. (a) Is it mediated by pancreatic hormones such as glucagon or insulin? (b) Is it mediated by cyclic AMP in the liver? (c) Does serotonin act directly on the liver? These questions still remain to be elucidated. It serotonin acts directly or via adenylyl cyclase in the liver, this might suggest a regulatory mechanism of indole amines in peripheral tissues. We are not sure yet whether the phenomenon reported in this paper represents a physiological role of indole amines, or it simply represents a pharmacological effect of serotonin exogenously administered. Indole amines have been shown to exhibit a number of actions; such compounds may induce or suppress some enzymes in tissues, resulting in metabolic changes. Such a possibility would represent another biochemical role of the biogenic amines.

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