[Chem. Pharm. Bull. 29(10)2940—2948(1981)]

Effect of *dl*-3-Pyridylalanine on Serotonin Concentration and Tryptophan-Serotonin Metabolizing Enzymes in Rats

Hiroshi Shimeno,* Yoko Fukumoto, Akihisa Toda and Atsuo Nagamatsu

Faculty of Pharmaceutical Sciences, Fukuoka University, 11 Nanakuma, Nishi-ku, Fukuoka, 814-01, Japan

(Received March 20, 1981)

A single administration of *dl*-3-pyridylalanine (3-PA, 100 mg/kg) significantly increased brain and serum serotonin (5-HT) concentrations without affecting the other tissue 5-HT concentrations (liver, kidney, spleen and small intestine) in male Wistar rats. The increase in brain 5-HT upon 3-PA administration was maintained for a long time (at least 96 h). Chronic administration of 3-PA (100 mg/kg/d) produced no change in brain 5-HT concentration as compared with that found in animals given a single dose of 3-PA. 3-PA decreased liver tryptophan pyrrolase (TP) activity and increased free tryptophan concentration in the serum. 3-PA hardly affected tryptophan 5-hydroxylase, 5-hydroxy-L-tryptophan (5-HTP) decarboxylase and monoamine oxidase (MAO) activities in the brain and liver. These findings suggest that the increase in brain 5-HT concentration upon administration of 3-PA occurs *via* the inhibition of liver TP, and an increase in free tryptophan concentration in the serum may be the causative factor for the increased brain 5-HT concentration.

In contrast with 3-PA, 5-HTP (50 mg/kg, 10 mg/kg) increased the 5-HT concentrations of all tissues studied, and the brain 5-HT concentration fell to control (saline-treated) levels within several hours after the administration of 5-HTP (10 mg/kg, 6 h; 50 mg/kg, 24 h). 5-HTP decreased brain tryptophan 5-hydroxylase and 5-HTP decarboxylase activities, and increased brain MAO and liver TP activities at 2 h after the administration.

Keywords——*dl*-3-pyridylalanine; 5-hydroxy-L-tryptophan; increasing effect on brain serotonin; serum tryptophan; tryptophan pyrrolase; tryptophan 5-hydroxylase; 5-hydroxy-L-tryptophan decarboxylase; monoamine oxidase

Administration of tryptophan to rats results in an increased synthesis of the neurotransmitter serotonin (5-HT) in the brain.[1] This suggests that tryptophan 5-hydroxylase (EC 1. 14. 16. 4), the rate-limiting enzyme in the pathway of 5-HT synthesis, in the brain is normally unsaturated with tryptophan and that the synthesis of 5-HT in the brain is dependent upon the concentration of brain tryptophan; this would be predicted from its K_m value *in vitro*.[2] Recent work has shown that the increased activity of liver tryptophan pyrrolase (TP, EC 1. 13. 11. 11) after the injection of corticosteroids into rats is followed by a decrease in the concentration of 5-HT in the brain.[3] These findings suggest that the changes in brain 5-HT metabolism may be associated with altered peripheral tryptophan metabolism.

We have previously reported that *dl*-3-pyridylalanine (3-PA) decreased liver TP activity in rats.[4] The present paper describes the comparative effects of 3-PA and L-5-hydroxytryptophan (5-HTP) on 5-HT concentration and on the activities of some tryptophan-5-HT metabolizing enzymes in rats. A possible mechanism for the increase of brain 5-HT by 3-PA is discussed.

Experimental

Chemicals——3-PA, mp 261—262°C, was synthesized by the method of Nieman *et al.*[5] 5-HTP and DL-6-methyl-5,6,7,8-tetrahydropterine dihydrochloride (6-MPH_4) were obtained from Nakarai Chem. Co., Kyoto. Bovine blood hematin and serotonin creatinine sulfate were purchased from E. Merck AG., Darmstadt, F.R.G. Bovine liver catalase and L-tryptophan were from P-L Biochem., Milwaukee, Wis., U.S.A. and Kyowa Hakko Kogyo Co., Tokyo, respectively. Other chemicals were of the purest grade available from Wako Junyaku Kogyo Co., Osaka.

Animals——Male Wistar rats (130—170 g, Kyudo Co., Kumamoto) were used. They were subcutaneously injected with a solution of 3-PA or 5-HTP in 0.9% NaCl. Control animals were injected with saline alone. When the drug was given repeatedly, subcutaneous injection was carried out once daily. All animals were deprived of food, but not water, for 19 h before sacrifice. They were killed by decapitation at the same time of day (14: 00—14: 30) to minimize the influence of possible diurnal variations. Blood was collected and allowed to clot for a few minutes at 37°C. For the measurement of free tryptophan in the serum, the protein-bound fraction was removed from a portion of each serum sample by ultrafiltration with an Amicon PM-10 membrane. The ultrafiltrate and serum were stored at −20°C until required for assay. The brain and the other tissues were dissected out rapidly, washed in ice-cold 0.9% NaCl, blotted dry and stored at 0°C until required tor assay.

Analytical Methods

5-HT——Whole brain, liver, kidney and spleen 5-HT were determined fluorometrically by the method of Barchas *et al.*[6] The small intestine was homogenized in 7 vol (v/w) of 0.1 N HCl–10% EDTA (9: 1) mixture at 4°C for 40 s with a Biotron homogenizer. The homogenizer was rinsed with 7 vol of the mixture. The homogenate and rinse were combined, and centrifuged at 30000 × *g* for 15 min at 4°C. The supernatant (2.0 ml) was diluted to 5.0 ml with 0.1 N HCl before the determination of 5-HT. Serum 5-HT was extracted with 0.4 N $HClO_4$ (5 ml/500 μl serum, containing 0.2 ml of 10% EDTA), centrifuged at 30000 × *g* for 15 min at 4°C and assayed by the method described above.

Tryptophan—Total and free (ultrafiltrable) tryptophan in the serum were measured fluorometrically by the method of Denckla and Dewey,[7] as modified by Bloxam and Warren.[8]

Tryptophan 5-Hydroxylase——Rat brain was homogenized in 2.5 vol of 0.05 M Tris–HCl (pH 7.4) containing 0.002 M dithiothreitol (DTT) at 4°C. The homogenate was centrifuged at 40000 × *g* for 20 min at 4°C and the supernatant was used as an enzyme source. The enzyme activity was assayed by the method of Friedman *et al.*[2] with a slight modification. The incubation mixture (total vol, 1.0 ml) contained 50 μmol of Tris–HCl (pH 7.4), 0.2 μmol of DTT, 6000 units of catalase, 0.2 μmol of 6-MPH_4, and 500 μl of enzyme preparation. After incubation at 37°C for 15 min with shaking, the reaction was stopped by the addition of 200 μl of 6 N $HClO_4$. Precipitated protein was removed by centrifugation at 2500 rpm for 10 min and 800 μl of the supernatant was added to 2.0 ml of 8 N HCl. The fluorescence of the solution was measured at 295 nm/565 nm (uncorrected). The amount of 5-HTP formed was calculated from a standard curve of 5-HTP run in parallel with each assay.

5-HTP Decarboxylase (EC 4. 1. 1. 28)——Tissues were homogenized in 3 vol (v/w) of H_2O and the homogenate was centrifuged at 35000 × *g* for 30 min at 4°C. The supernatant was used as an enzyme source. The enzyme activity was determined in terms of the formation of 5-HT from 5-HTP according to the method of Lovenberg.[9]

Monoamine Oxidase (MAO, EC 1. 4. 3. 4)——MAO activity was determined in terms of the rate of degradation of 5-HT according to the method of Karki *et al.*[10] with a modification. Tissues were homogenized in 0.9% NaCl (10 vol for brain, 200 vol for liver) at 4°C. The homogenate (1.0 ml) was pipetted into incubation flasks containing 0.3 M phosphate buffer (0.3 ml, pH 7.4), and water was added to a volume of 2.3 ml. Samples were preincubated at 37°C for 20 min, 5-HT was added to a final concentration of 14 μg/ml, and the mixture was incubated for another 30 min. The reaction mixture was mechanically shaken with 15 ml of acidified *n*-butanol for 15 min. After centrifugation for 5 min at 3000 rpm, 12.5 ml of the supernatant was pipetted into a 50 ml glass stoppered tube and shaken for 15 min with 25 ml of *n*-heptane and 2.0 ml of 0.1 N HCl. The phases were separated by centrifugation as before. The aqueous phase (2.0 ml) was added to 0.7 ml of conc. HCl and the fluorescence was measured at 295 nm/565 nm. The amount of 5-HT was calculated from a standard curve of 5-HT run in parallel with each assay.

Tryptophan Pyrrolase——TP activity in liver homogenate was determined by the method of Kewitz and Wagner[11] with the addition of 2×10^{-6} M hematin to the reaction mixture.

All results were examined by using Student's *t* test.

Results

Effect of 3-PA on Brain and Serum 5-HT Concentrations

The effect of 3-PA given at four dose levels on the concentration of 5-HT in rat brain and serum is shown in Fig. 1-a. There was a dose-related increase in 5-HT concentration, this effect being statistically significant at the 100 and 150 mg/kg doses but not at the 25 and 50 mg/kg doses of 3-PA. The degree of increase in brain 5-HT (16%) after 100 mg/kg of 3-PA was not statistically different from that (19%) after 150 mg/kg. In this experiment, 5-HT was measured at 24 h after the administration of 3-PA.

The time courses of changes in brain and serum 5-HT concentrations after a single administration of 3-PA (100 mg/kg) are shown in Fig. 1-b. 3-PA caused an increase of 5-HT in the

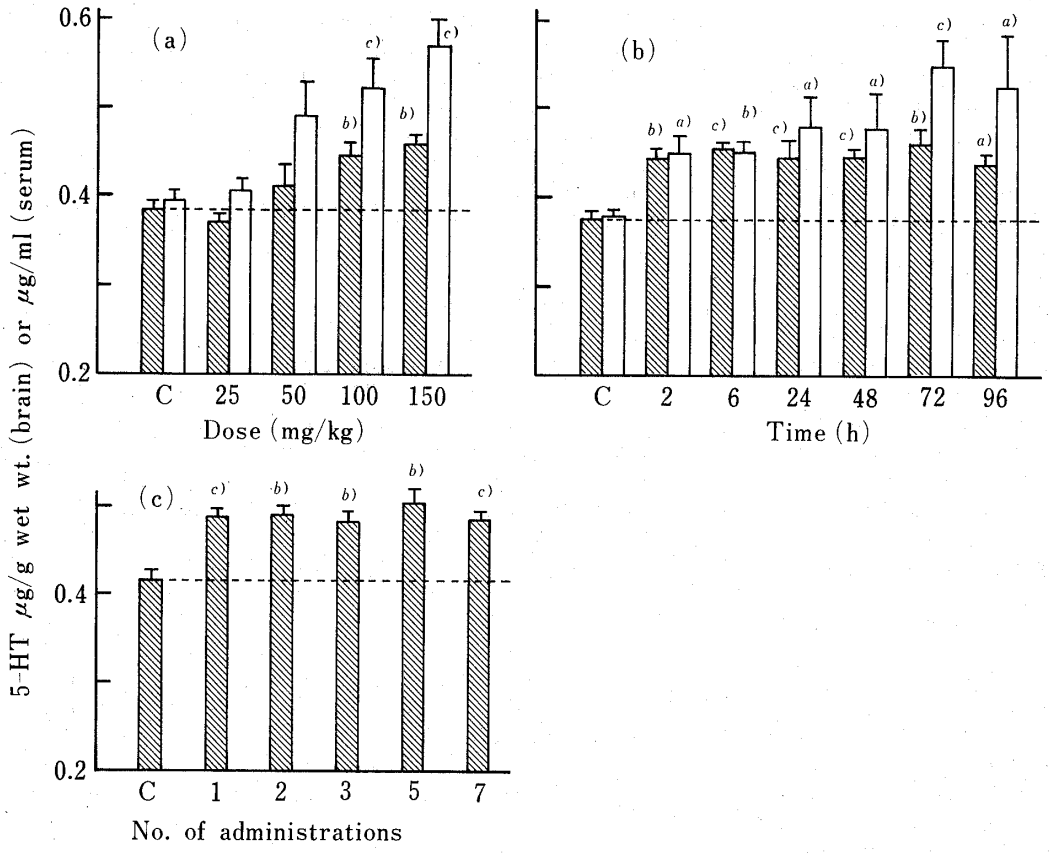

Fig. 1. Effect of *dl*-3-Pyridylalanine on Rat Brain and Serum Serotonin (5-HT) Concentrations

Hatched columns, brain 5-HT; open columns, serum 5-HT.
Vertical bars represent the means ± S.E. (6 animals per group).
Significance of difference from controls: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

brain at 2 h after administration, and it was still apparent at 96 h. Although the increases in brain 5-HT level after 3-PA administration were small (16—22%), they were statistically significant as compared with brain 5-HT levels in control (saline-treated) animals. The time course of changes in 5-HT concentration in the serum was similar to that in the brain.

The effect of repeated daily administrations of 3-PA at the dose of 100 mg/kg/d is shown in Fig. 1-c. The concentration of brain 5-HT was measured at 24 h after the last injection. In all groups of rats administered 3-PA, there was a significant increase in the concentration of brain 5-HT. However, repeated administrations of 3-PA did not produce any further increase in brain 5-HT concentration as compared with that found in animals given a single dose of 3-PA.

Effect of 3-PA on Liver, Kidney, Spleen and Small Intestine 5-HT Concentrations

As presented in Table I, the liver, kidney and spleen 5-HT concentrations after a single administration of 3-PA (100 mg/kg) were not different from those of saline-treated animals. The small intestine 5-HT levels increased slightly 72 and 96 h after the injection of 3-PA, but not significantly. In these tissues, 5-HT concentrations were hardly affected even at a dose of 150 mg/kg.

The effect of repeated daily administrations of 3-PA (100 mg/kg/d) on various tissue 5-HT concentrations is shown in Table II. The chronic administration of 3-PA (7 d) resulted in a significant elevation of the small intestine 5-HT level. However, the liver, kidney and spleen 5-HT levels were unchanged by chronic 3-PA administration.

TABLE I. Effect of *dl*-3-Pyridylalanine (100 mg/kg) on Serotonin (5-HT) Concentration in Various Tissues

Time (h)	Liver	5-HT μg/g tissue wet wt. Kidney	Spleen	Small intestine
Control	0.591±0.015	0.270±0.012	3.66±0.19	5.56±0.13
2	0.602±0.013	0.283±0.005	3.80±0.17	5.53±0.13
6	0.558±0.024	0.262±0.008	3.37±0.24	5.76±0.09
24	0.601±0.020	0.281±0.003	3.24±0.17	5.98±0.21
48	0.562±0.020	0.298±0.005	3.73±0.14	6.10±0.21
72	0.563±0.028	0.259±0.010		6.32±0.33
96	0.562±0.026	0.257±0.006		6.19±0.28

Tissue 5-HT concentrations were determined at the times indicated.
Values represent the means±S.E. for 6 animals per group.

TABLE II. Effect of Repeated Administrations of *dl*-3-Pyridylalanine (100 mg/kg/d) on Serotonin (5-HT) Conce ntration in Various Tissues

No. of administrations	Liver	5-HT μg/g tissue wet wt. Kidney	Spleen	Small intestine
Control	0.539±0.015	0.326±0.008	3.41±0.30	5.87±0.05
1	0.510±0.021	0.344±0.006	3.15±0.11	5.69±0.10
2	0.495±0.023	0.313±0.008	4.00±0.34	5.80±0.17
3	0.556±0.025	0.307±0.006	3.63±0.15	5.85±0.06
5	0.553±0.055	0.336±0.012	3.31±0.21	6.39±0.26
7	0.555±0.045	0.307±0.008	3.19±0.15	7.26±0.38[a)]

Tissue 5-HT concentrations were determined at 24 h after the last injection.
Values represent the means±S.E. for 6 animals per group.
Significance of difference from controls: *a*) $p<0.01$.

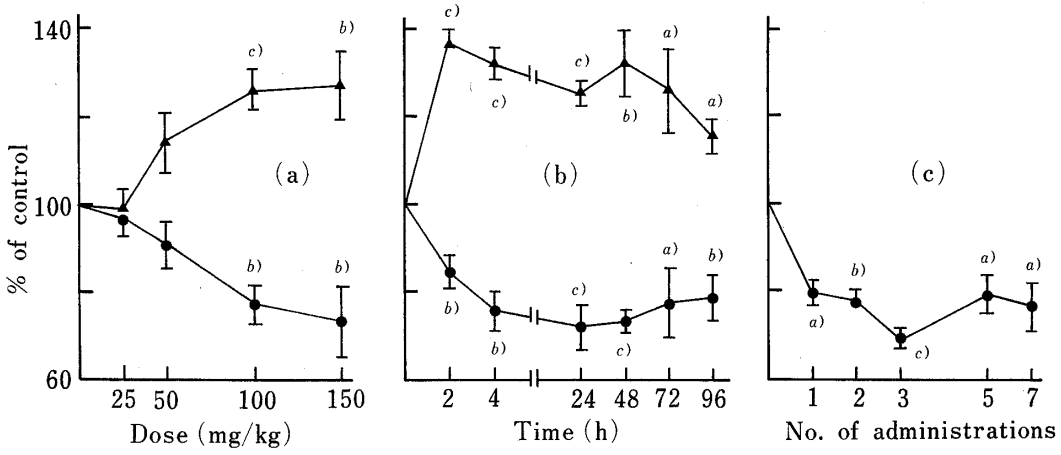

Fig. 2. Effect of *dl*-3-Pyridylalanine on Liver Tryptophan Pyrrolase (TP, ●) Activity and Serum Free Tryptophan (Trp, ▲) Concentration

Points represent the means±S.E. (6 animals per group).
Significance of difference from controls: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.
Control values are as follows:

	(a)	(b)	(c)
TP (kynurenine μmol/g wet wt./h)	7.56±0.70	9.28±0.11	9.13±0.59
Trp (μg/ml)	2.15±0.05	2.46±0.05	

Effect of 3-PA on Tryptophan-5-HT Metabolizing Enzymes and Serum Free Tryptophan

In order to clarify the mechanism of the 5-HT increase in the brain induced by 3-PA, the activities of enzymes involved in the metabolism of tryptophan and 5-HT were determined in the brain and liver of animals administered 3-PA. Furthermore, the concentration of free tryptophan in the serum was measured at the same time.

a) Liver TP and Serum Free Tryptophan——Fig. 2-a shows the effect of 3-PA on TP activity in the liver and on free tryptophan in the serum at 24 h after administration. Liver

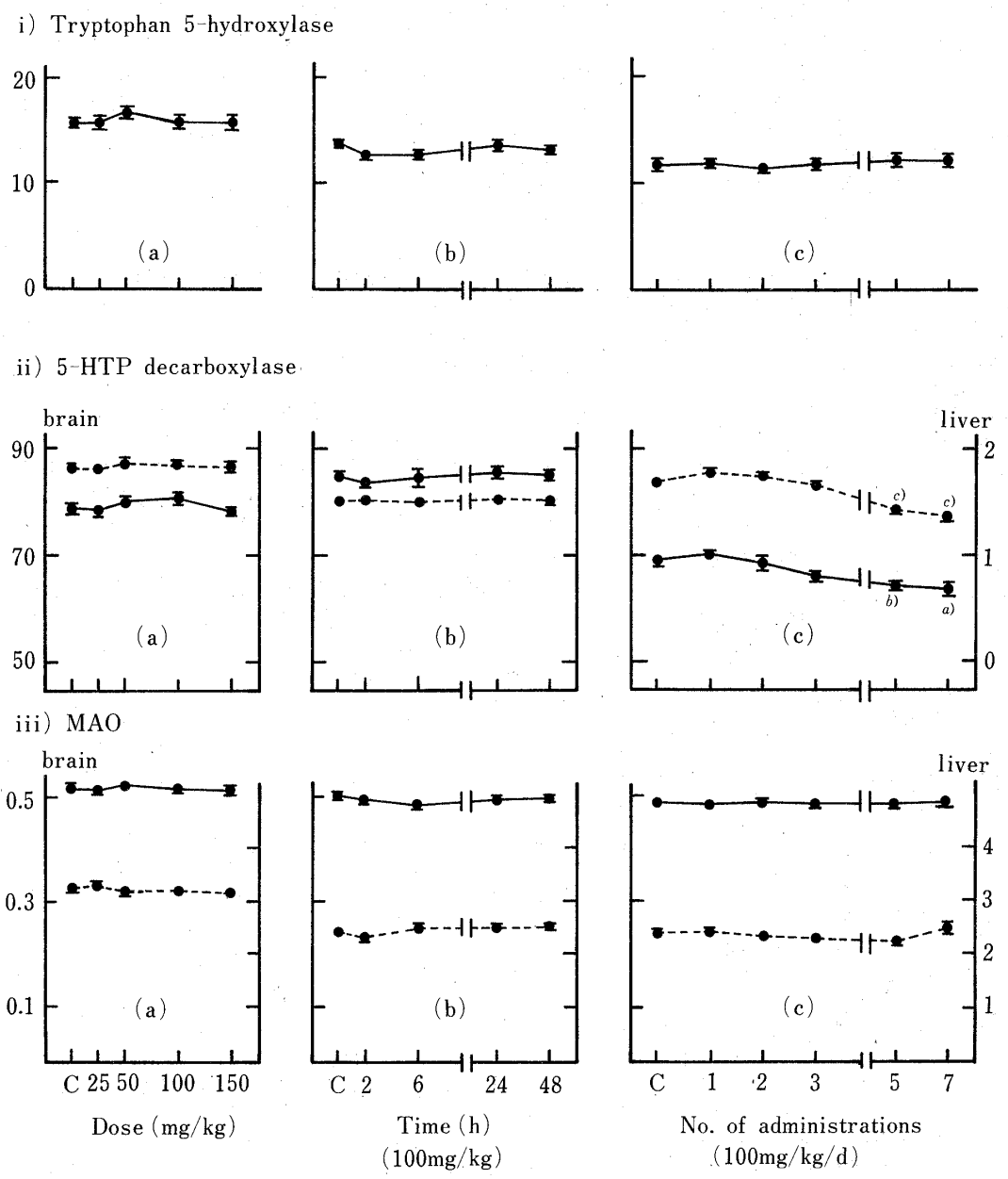

Fig. 3. Effect of *dl*-3-Pyridylalanine on the Activities of Three Enzymes involved in Serotonin (5-HT) Metabolism in the Brain (●——●) and Liver (●----●)

Points represent the means±S.E. (6 animals per group).
Significance of difference from controls: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.
Enzyme activities are expressed as follows:
i) Tryptophan 5-hydroxylase: brain, 5-HTP nmol/g wet wt./15 min.
ii) 5-Hydroxy-L-tryptophan (5-HTP) decarboxylase: brain, 5-HT μg/g wet wt./h; liver, 5-HT mg/g wet wt./h
iii) Monoamine oxidase (MAO): brain, 5-HT mg/g wet wt./30 min; liver, 5-HT mg/g wet wt./30 min.

TP activity decreased at all doses and the decrease was significant at 100 and 150 mg/kg. On the other hand, the concentration of free tryptophan in the serum significantly increased at the doses of 100 and 150 mg/kg.

The time courses of the effect of a single dose of 3-PA (100 mg/kg) on TP activity in the liver and on free tryptophan in the serum are shown in Fig. 2-b. Two hours after the 3-PA administration, a significant decrease in liver TP activity and a significant increase of the serum free tryptophan level were observed. These effects of 3-PA were maintained throughout the duration of the experiment.

The effect of repeated administrations of 3-PA (100 mg/kg) on liver TP activity is shown in Fig. 2-c. The enzyme activity was determined at 24 h after the last injection. Liver TP activity in chronically 3-PA-treated animals was not statistically different from that determined in animals given a single injection of 3-PA.

b) Brain Tryptophan 5-Hydroxylase——Fig. 3-i shows that single and repeated administrations of 3-PA did not cause a statistically significant change in brain tryptophan 5-hydroxylase activity.

c) Brain and Liver 5-HTP Decarboxylase——No significant changes in brain and liver 5-HTP decarboxylase activities were found in animals given a single dose of 3-PA (Fig. 3-ii-a and b). On the other hand, the chronic administration of 3-PA (100 mg/kg/d, for 5 and 7 d) produced a significant decrease in the brain and liver enzyme activities as compared with control animals (Fig. 3-ii-c). The decrease of the enzyme activity in the brain was about one-half of that found in the liver (21% for 7 d).

d) Brain and Liver MAO——Brain and liver MAO activities were not influenced by 3-PA administration (Fig. 3-iii).

Effect of 5-HTP on Brain and Serum Concentrations

In order to compare its effect with that of 3-PA, a single dose of 5-HTP (10 or 50 mg/kg)

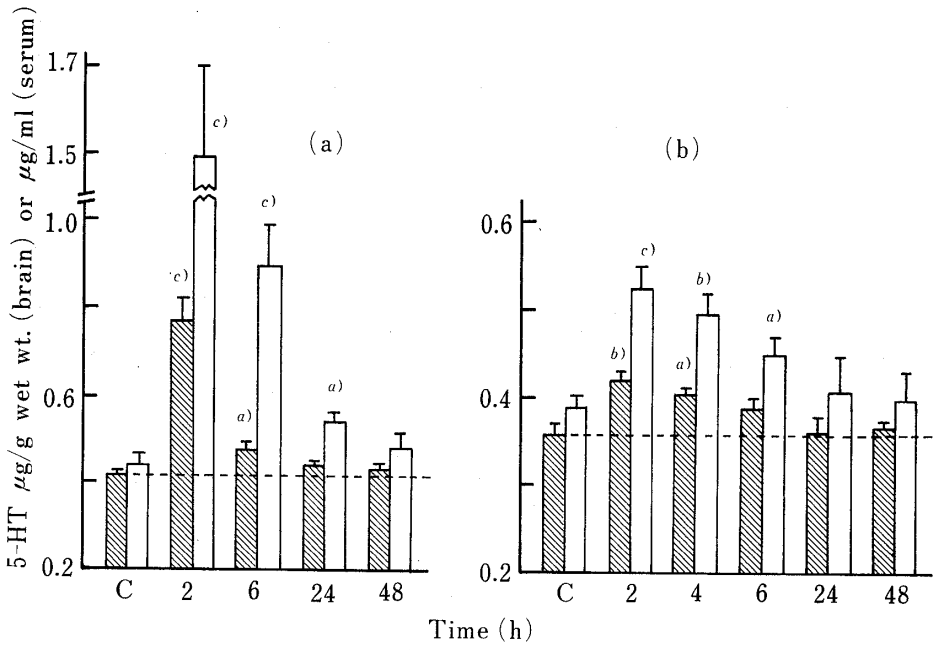

Fig. 4. Effect of 5-Hydroxy-L-tryptophan on Rat Brain and Serum Serotonin (5-HT) Concentrations

Rats were injected with 50 mg/kg in experiment (a) and 10 mg/kg in experiment (b). Vertical bars represent the means±S.E. (6 animals per group). Hatched columns, brain 5-HT; open columns, serum 5-HT. Significance of difference from controls: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

was given to rats, and 5-HT concentrations in the brain and serum were determined at various times after the administration. As shown in Fig 4, the administration of 5-HTP significantly increased the concentration of brain 5-HT after 2 h. The increase reached by 117% and 182% of the control 5-HT levles after administration of 10 mg/kg and 50 mg/kg, respectively. In contrast with the case of 3-PA, the increased brain 5-HT levels rapidly returned to the control levels (6 h for 10 mg/kg, 24 h for 50 mg/kg). The time course of changes in serum 5-HT concentration was similar to that in the brain, although the increase in serum 5-HT was considerably greater than that in brain 5-HT.

Effect of 5-HTP on Liver, Kidney, Spleen and Small Intestine 5-HT Concentrations

The time courses of changes in various tissue 5-HT concentrations after a single administration of 5-HTP (50 mg/kg) are shown in Fig. 5. In contrast with the effect of 3-PA, the administration of 5-HTP significantly increased the concentration of 5-HT in all tissues examined. At 2 h after the administration, the kidney 5-HT level was 44 times the control, while the liver, spleen and small intestine 5-HT levels reached 199, 237 and 136% of the control values, respectively. Kidney 5-HT concentration returned to the control level 48 h after the administration. On the other hand, the liver, spleen and small intestine 5-HT concentrations remained significantly higher than those in control animals throughout this experiment.

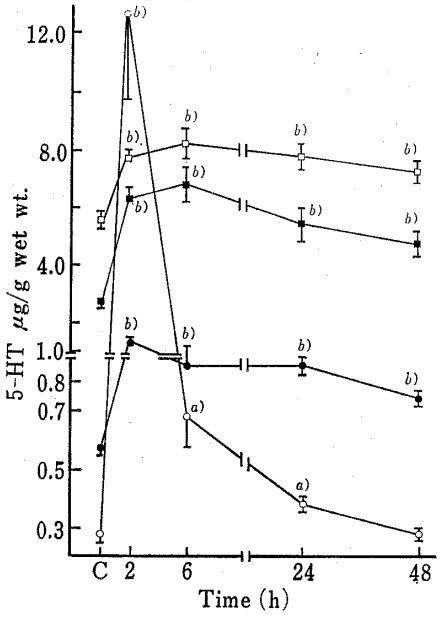

Fig. 5. Effect of 5-Hydroxy-L-tryptophan on the Liver (●), Kidney (○), Spleen (■) and Small Intestine (□) Serotonin (5-HT) Concentrations

Points represent the means±S.E. (6 animals per group).
Significance of difference from controls: *a*) $p<0.01$, *b*) $p<0.001$.

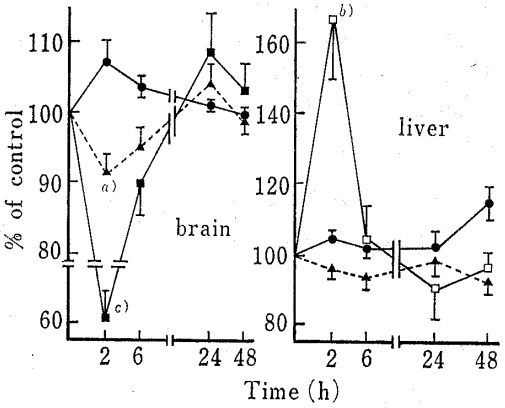

Fig. 6. Effect of 5-Hydroxy-L-tryptophan (5-THP) on Brain and Liver Tryptophan-Serotonin Metabolizing Enzymes

Points represent the means±S.E. (6 animals per group).
Significance of difference from controls: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.
Control values are as follows:
Brain
■: Tryptophan 5-hydroxylase (5-HTP nmol/g wet wt./15 min) 15.4±0.6.
▲: 5-HTP decarboxylase (serotonin μg/g wet wt./h) 86.8±1.5.
●: Monoamine oxidase (serotonin mg/g wet wt./30 min) 0.459±0.006.
Liver
□: Tryptophan pyrrolase (kynurenine μmol/g wet wt./h) 7.70±0.62.
▲: 5-HTP decarboxylase (serotonin mg/g wet wt./h) 1.89±0.03.
●: Monoamine oxidase (serotonin mg/g wet wt./30 min) 2.77±0.20.

Effect of 5-HTP on Tryptophan-5-HT Metabolizing Enzymes

The activities of several enzymes involved in the metabolism of tryptophan and 5-HT in the brain and liver were determined at various times after a single administration of 5-HTP (50 mg/kg). The activity of brain tryptophan 5-hydroxylase was markedly lower at 2 h after the administration and then gradually returned to the control level. Brain 5-HTP decarboxyl-

ase activity was also significantly lower at 2 h, whereas brain MAO activity increased as compared with that in control animals (not statistically significant). At 6 h after the administration, the two enzyme activities were not different from those in the control group (Fig. 6). In the liver, the activities of these two enzymes were hardly affected by 5-HTP administration. However, the TP activity increased to 170% at 2 h after 5-HTP administration then rapidly declined to the control level.

Discussion

In the present study, an increase in brain 5-HT was observed along with a decreased TP activity in the liver and an increased level of free tryptophan in the serum upon administration of 3-PA. *In vitro* determination of the K_m of tryptophan 5-hydroxylase suggested that the enzyme in the brain is not normally saturated with tryptophan.[2] This implies that an increase in the availability of tryptophan to the brain would result in an increase of 5-HTP synthesis. There is no evidence that the decarboxylation of 5-HTP to 5-HT is rate-limiting, and there is a considerably greater activity of 5-HTP decarboxylase than of tryptophan 5-hydroxylase in the brain.[12] Therefore, brain tryptophan concentration may play an important role in 5-HT synthesis. There is a considerable amount of evidence in favor of this concept, as well as evidence of a direct relationship between the concentration of free tryptophan in the serum (or plasma) and that of brain tryptophan.[13] Also, the induction of liver TP by corticosteroid treatment is well known to result in a decrease in brain 5-HT concentration.[3,13c] Therefore, we infer that the increase of brain 5-HT by 3-PA may occur *via* the inhibition of liver TP and an increase in free tryptophan in the serum.

3-PA hardly affected the activities of three enzymes involved in the synthesis and catabolism of 5-HT (tryptophan 5-hydroxylase, 5-HTP decarboxylase and MAO). This also supports the hypothesis that a decrease in TP activity induced by 3-PA is a causative factor for the increased brain 5-HT concentration. Since 3-PA and tryptophan have similar chemical structures (heterocyclic α-amino acids), liver TP may be competitively inhibited by 3-PA.

In contrast with 3-PA, the administration of 5-HTP produced an increase in 5-HT concentration not only in the brain but also in all tissues examined. Unlike tryptophan, 5-HTP is a direct precursor of 5-HT *in vivo* and is decarboxylated to 5-HT without the action of the key enzyme in the normal synthetic pathway of 5-HT, tryptophan 5-hydroxylase. Therefore, a non-physiological distribution of 5-HT in tissues and cells would be produced by the administration of 5-HTP. In animals administered 5-HTP, a rapid increase and subsequent decrease in brain 5-HT concentration were observed in association with the changes in brain tryptophan 5-hydroxylase and 5-HTP decarboxylase activities. These findings suggest that a feedback inhibition by 5-HT is involved in the regulation of brain 5-HT synthesis, as well as the availability of tryptophan. However, 3-PA administration did not elicit feedback inhibition in brain 5-HT synthesis. It is assumed that the increase in brain 5-HT concentration after 3-PA administration is insufficient to elicit the feedback inhibition.

There was a significant increase in liver TP activity after 5-HTP administration. 5-HTP is known to cause an increase in plasma and adrenal corticosterone.[14] Also, the chemical structure of 5-HTP is similar to that of tryptophan, which is known to produce a substrate-type enhancement of TP activity.[15] These findings imply that the increase in TP activity upon 5-HTP administration may be based on a hormonal and/or substrate-type mechanism.

Tryptophan has been used in the treatment of depressive illness[16] on the basis of evidence that brain 5-HT or its turnover is reduced in this disorder.[16,17] However, the efficacy of tryptophan is still controversial.[18] It has been shown recently that tryptophan might be more effective as an antidepressant when given with a TP inhibitor.[19] These results suggest that a TP inhibitor, 3-PA, may increase the level of brain tryptophan and prolong the period of elevated concentration of brain 5-HT in animals (or humans) given tryptophan. Therefore, 3-PA may be capable of producing changes in some patients with neuropsychiatric disorders.

References and Notes

1) *a*) D. Eccleston, G.W. Ashcroft, and T.B.B. Crawford, *J. Neurochem.*, **12**, 493 (1965); *b*) J.D. Fernstrom and R.J. Wurtman, *Science*, **173**, 149 (1971).
2) P.A. Friedman, A.H. Kappelman, and S. Kaufman, *J. Biol. Chem.*, **247**, 4165 (1972).
3) *a*) A.R. Green, T.L. Sourkes, and S.N. Young, *Brit. J. Pharmacol.*, **53**, 287 (1975); *b*) A.R. Green, H.F. Woods, P.J. Knott, and G. Curzon, *Nature* (London), **255**, 170 (1975).
4) H. Shimeno, T. Kuroiwa, and A. Nagamatsu, *Yakugaku Zasshi*, **100**, 1078 (1980).
5) C. Nieman, R.N. Lewis, and J.T. Hays, *J. Am. Chem. Soc.*, **64**, 1678 (1942).
6) J. Barchas, E. Erdelyi, and P. Angwin, *Anal. Biochem.*, **50**, 1 (1972).
7) W.D. Denckla and H.K. Dewey, *J. Lab. Clin. Med.*, **69**, 160 (1967).
8) D.L. Bloxam and W.H. Warren, *Anal. Biochem.*, **60**, 621 (1974).
9) W. Lovenberg, "Methods in Enzymology," Vol. 17(B), ed. by H. Tabor and C.W. Tabor, Academic Press, Inc., New York, 1971, p. 652.
10) N. Karki, R. Kuntzman, and B.B. Brodie, *J. Neurochem.*, **9**, 53 (1962).
11) H. Kewitz and H. Wagner, *Arzneim.-Forsch.*, **15**, 1 (1965).
12) A. Ichiyama, S. Nakamura, Y. Nishizuka, and O. Hayaishi, *J. Biol. Chem.*, **245**, 1699 (1970).
13) *a*) G. Curzon and P.J. Knott, *Brit. J. Pharmacol.*, **50**, 197 (1974); *b*) A.A.-B. Badawy and M. Evans, *Biochem. J.*, **160**, 315 (1976); *c*) A.A.-B. Badawy, *Life Sci.*, **21**, 755 (1977).
14) *a*) F. Okada, Y. Saito, T. Fujieda, and I. Yamashita, *Nature* (London), **238**, 355 (1972); *b*) A. Kawa, T. Ariyama, Y. Yaniguchi, T. Kamisaki, and T. Kanehisa, *Acta Endocrinol.*, **89**, 432 (1978).
15) *a*) W.E. Knox, *Brit. J. Exp. Path.*, **32**, 462 (1951); *b*) O. Greengard and P. Feigelson, *J. Biol. Chem.*, **236**, 158 (1961).
16) *a*) A. Coppen, D.M. Shaw, B. Herzberg, and R. Maggs, *Lancet*, **2**, 1178 (1967); *b*) A. Coppen, A.J. Prange Jr., P.C. Whybrow, and R. Noguera, *Arch. Gen. Psychiat.*, **26**, 474 (1972).
17) *a*) F.K. Goodwin and R.M. Post, *Adv. Biochem. Psychopharmacol.*, **11**, 341 (1974); *b*) K.G. Lloyd, I.J. Farley, J.H.N. Deck, and O. Hornykiewicz, *ibid.*, **11**, 387 (1974).
18) *a*) D.L. Murphy, M. Baker, F.K. Goodwin, H. Miller, J. Kotin, and W.E. Bunney, *Psychopharmacology*, **34**, 11 (1974); *b*) K. Jensen, K. Fruensgaard, U.G. Ahlfors, T.A. Pihkanan, S. Toumikoski, E. Ose, S.J. Dencker, D. Lindberg, and A. Nagy, *Lancet*, **2**, 920 (1975); *c*) G. d'Elia, L. Hanson, and H. Raotma, *Acta Psychiat. Scand.*, **57**, 239 (1978).
19) *a*) M.H. Joseph, S.N. Young, and G. Curzon, *Biochem. Pharmacol.*, **25**, 2599 (1976); *b*) B. Shopsin, *Neuropsychobiology*, **4**, 188 (1978).