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Effect of Combined Administration of Tryptophan with Putative Tryptophan Pyrrolase Inhibitors, DL-3-Pyridylalanine, Allopurinol or Nicotinamide, on Brain Serotonin Concentration

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1) Single administration of tryptophan (25—200 mg/kg) to Wistar male rats resulted in a dose-related increase in liver tryptophan pyrrolase (TP) activity. The increase in brain serotonin (5-HT) concentration was not proportional to the dose given, and brain 5-HT concentration rapidly fell to the control (saline-treated) level or below. 2) The tryptophan-induced increase in liver TP activity was clearly prevented by DL-3-pyridylalanine (3-PA, 100 mg/kg). Serum free tryptophan and brain 5-HT concentrations increased more markedly and maintained higher levels after the combined administration of tryptophan with 3-PA than after a single administration of tryptophan. 3) Although allopurinol (20 mg/kg) reduced liver TP activity under control and tryptophan-treated conditions, it did not increase serum free tryptophan and brain 5-HT concentrations. 4) Nicotinamide also failed to alter the catabolism of tryptophan and the concentration of brain 5-HT in control or tryptophan-treated rats. 5) These data suggest that 3-PA would increase the therapeutic effect of tryptophan given to treat depressive illness, but that allopurinol and nicotinamide would not be suitable drugs for this purpose.

Keywords——DL-3-pyridylalanine; L-tryptophan; allopurinol; nicotinamide; increasing effect on brain serotonin; serum tryptophan; liver tryptophan pyrrolase

Tryptophan is the precursor of the neurotransmitter serotonin (5-HT). *In vitro* determination of the K_m value of tryptophan 5-hydroxylase (L-tryptophan, tetrahydropteridine : oxidoreductase (5-hydroxylating), EC 1.14.16.4, the first enzyme in the pathway of 5-HT synthesis) suggested that the enzyme in the brain is normally unsaturated with tryptophan.[1] This implies that an increase in the availability of tryptophan to the brain would result in an increase of 5-HT synthesis.[2] Tryptophan has been used for several years in the treatment of depression on the basis of evidence that brain 5-HT or its turnover is reduced in this disorder.[3] However, the efficacy of tryptophan in depression is still controversial.[4] A problem of tryptophan treatment is that large amounts of tryptophan are metabolized by tryptophan pyrrolase (L-tryptophan : oxygen 2,3-oxidoreductase, EC 1.13.11.11, TP) to kynurenine in the liver, so that relatively little is available for the synthesis of brain 5-HT.[5] Therefore, inhibition of the activity of liver TP, which is induced by its substrate, tryptophan,[6] may increase 5-HT synthesis in the brain.

We have previously reported that DL-3-pyridylalanine (3-PA) significantly increased brain 5-HT concentration in rats.[7] This compound decreased liver TP activity and increased serum free tryptophan concentration. On the basis of these findings, we suggested that the increase in brain 5-HT concentration upon the administration of 3-PA occurred *via* the inhibition of liver TP, and an increase in serum free tryptophan concentration might be the causative factor of the increased brain 5-HT concentration. Allopurinol (4-hydroxy-

pyrazolo[3,4-*d*]pyrimidine) and nicotinamide have been suggested to have potential use as TP inhibitors and might be useful in conjunction with tryptophan to treat depression.[8]

We have examined the effect of combined administration of tryptophan with 3-PA, allopurinol or nicotinamide on brain 5-HT concentration, serum free tryptophan concentration and liver TP activity. We now report that 3-PA is capable of reducing the induction of TP by tryptophan and, because of this, can efficiently increase the brain 5-HT concentration after a tryptophan load. The data are discussed in relation to the possible treatment of depression.

Materials and Methods

Chemicals——3-PA, mp 261—262 °C, was synthesized by the method of Nieman *et al.*[9] Bovine blood hematin and 5-HT creatinine sulfate were purchased from E. Merck AG., Darmstadt, F.R.G. Allopurinol and nicotinamide were obtained from Nakarai Chem. Co., Kyoto. Tryptophan was from Kyowa Hakko Kogyo., Tokyo. Other chemicals were of the purest grade available from Wako Junyaku Co., Osaka.

Animals and Administration——Male Wistar rats (130—170 g, Kyudo Co., Kumamoto) were used. 3-PA was dissolved in 0.9% (w/v) NaCl. Tryptophan and allopurinol were dissolved in the minimum amounts of 1 N NaOH and diluted to 20 ml with 0.9% NaCl after the pH had been adjusted 7.5 with 1 N HCl. Each animal received a subcutaneous injection of 3-PA, tryptophan or allopurinol solution. Control animals were injected with saline alone. Nicotinamide was administered to fed rats at the concentration of 1.0 g/l in the drinking water (available *ad libitum*) for 7 d according to the method of Badawy *et al.*[10] The corresponding control animals were given an equal quantity of drinking water.

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 brain was dissected out rapidly, washed in ice-cold 0.9% NaCl, blotted dry and stored at −20 °C until required for assay.

Determination of Brain 5-HT Concentration——Whole brain 5-HT was fluorometrically determined by the method of Barchas *et al.*[11]

Assay of Liver TP——The activity of the enzyme was determined in liver homogenate by measuring the formation of kynurenine from tryptophan either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of 2 μM hematin.[12] The apoenzyme activity was calculated as the difference.

Determination of Serum Free Tryptophan Concentration——Free (ultrafiltrable) tryptophan in the serum was measured fluorometrically by the method of Denkla and Dewey,[13] as modified by Bloxam and Warren.[14]

Statistical Analysis——Statistical analysis of results was performed by the use of Student's *t*-test.[15] p values of <0.05 were accepted as significant.

Results

Effects of Administration of Various Doses of Tryptophan on Liver TP Activity and Brain 5-HT Concentration

Figure 1-i shows that at 2 h after the administration of tryptophan, liver TP holoenzyme and total enzyme activities were significantly increased at all doses. The increase was approximately proportional to the dose given. Brain 5-HT concentration was not significantly altered by the 25 mg/kg dose, but was increased by the other three doses. However, the degree of increase in brain 5-HT after 200 mg/kg of tryptophan was lower than that after 100 mg/kg.

At 6 h after the administration of 200 mg/kg of tryptophan, brain 5-HT concentration was significantly decreased as compared with that in control (saline-treated) animals (Fig. 1-ii). Only a 50 mg/kg dose of tryptophan produced a significant increase in brain 5-HT concentration at this time. As shown in Fig. 1-ii, the degree of increase in liver TP activity at 6 h after the administration of tryptophan was considerably lower than that at 2 h. In the case of a 100 or 200 mg/kg dose of tryptophan, significant increases in the TP holoenzyme and total enzyme activities were still observed as compared with the control activities. These

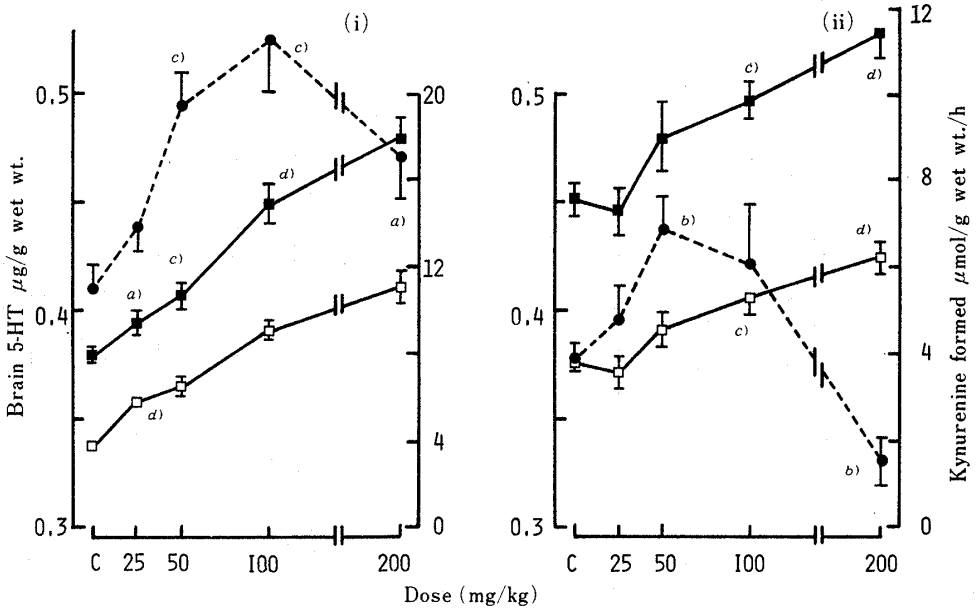

Fig. 1. Effect of Tryptophan on Rat Brain 5-HT Concentration and Liver TP Activity

Rats received at 2 h (i) or 6 h (ii) before death a subcutaneous injection of saline or various doses of tryptophan (25—200 mg/kg). Brain 5-HT concentration (●---●) was determined as described in Materials and Methods. Liver TP activity was determined as described in Materials and Methods either in the absence (holoenzyme activity, □—□) or in the presence (total enzyme activity, ■—■) of added hematin. Points represent the means ± S.E. (6 animals per group). Significance of difference from controls is indicated as follows; *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$.

findings suggest that high activity of liver TP prevents the increase in brain 5-HT concentration.

Effects of Combined Administration of Tryptophan with 3-PA on Brain 5-HT Concentration, Liver TP Activity and Serum Free Tryptophan Concentration

In this section, the time course of the effects of tryptophan on brain 5-HT concentration, liver TP activity and serum free tryptophan concentration was examined in detail. Furthermore, the effect of the combined administration of tryptophan with 3-PA was also investigated. The results are shown in Figs. 2—4.

a) Effects on Brain 5-HT Concentration——In agreement with previous results,[7] 3-PA caused a significant increase in brain 5-HT concentration at 24 h after the administration (Fig. 2).

The result in Fig. 2-i shows that no significant increase in brain 5-HT concentration was produced by a 25 mg/kg dose of tryptophan at any of the times examined. In contrast, the combined injection of tryptophan with 3-PA resulted in an apparent increase in brain 5-HT at all times as compared with that in rats administered tryptophan alone.

As shown in Fig. 2-ii, brain 5-HT concentration increased to 118% of the control at 1 h after the injection of 50 mg/kg of tryptophan, and then gradually returned to the control level (within 24 h). In the case of the combined administration with 3-PA, brain 5-HT increased to 143% of the control at 1 h after the administration. The increase reached a maximum at 2 h, and was still apparent at 24 h.

Brain 5-HT concentration in rats injected with 100 mg/kg of tryptophan increased to 129% of the control at 2 h, but fell to the control level at 6 h after the injection (Fig. 2-iii). On the other hand, the combined administration with 3-PA caused significant increases in brain 5-HT at all the times examined, as compared with the saline or tryptophan-treated group.

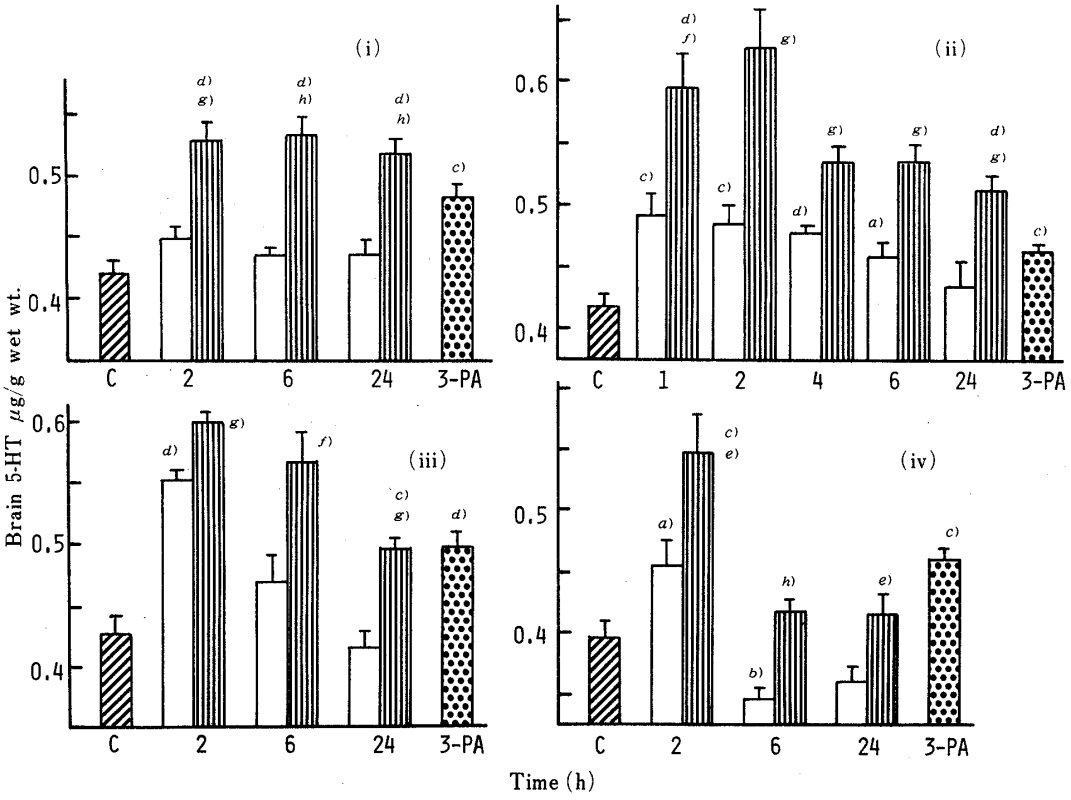

Fig. 2. Effects of Tryptophan Alone and in Combination with 3-PA on Rat Brain 5-HT Concentration

Rats were subcutaneously injected with various doses of tryptophan (i, 25 mg/kg; ii, 50 mg/kg; iii, 100 mg/kg; iv, 200 mg/kg) and sacrificed at the time indicated. At 24 h before the sacrifice, they had been injected with 3-PA (100 mg/kg) or saline. Brain 5-HT concentration was determined as described in Materials and Methods. Vertical bars represent the means ± S.E. (6 animals per group). ▨, control (saline-treated); □, tryptophan; ▤, tryptophan + 3-PA; ▩, 3-PA. Significance of differences from controls: *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$. Significance of differences from tryptophan group: *e*) $p<0.05$, *f*) $p<0.02$, *g*) $p<0.01$, *h*) $p<0.001$.

As shown in Fig. 2-iv, brain 5-HT concentration increased to 115% of the control at 2 h after a single administration of 200 mg/kg of tryptophan, whereas it decreased to 87% at 6 h. The decrease was significantly prevented by 3-PA.

b) Effects on Liver TP Activity and Serum Free Tryptophan Concentration——In order to clarify the mechanism of changes in brain 5-HT concentration induced by tryptophan and 3-PA, liver TP activity and serum free tryptophan concentration were determined at various times after single and/or combined injection of tryptophan and 3-PA. The results are shown in Figs. 3 and 4.

At 24 h after a single administration of 3-PA, liver TP holoenzyme and total enzyme activities were significantly decreased as compared with those in control rats (Fig. 3). The apoenzyme activity was not altered by 3-PA. 3-PA significantly increased the concentration of free tryptophan in the serum (Fig. 4).

Figure 3-i shows that at 2 h after single administration of 25 mg/kg of tryptophan, liver TP holoenzyme and total enzyme activities were increased to 154 and 122% of the control values, respectively. The increases were completely inhibited by 3-PA. On the other hand, serum free tryptophan concentration was not altered by a 25 mg/kg dose of tryptophan, but was significantly increased by combined administration with 3-PA at all the times examined (Fig. 4-i). The increase was 28% over the control at 24 h.

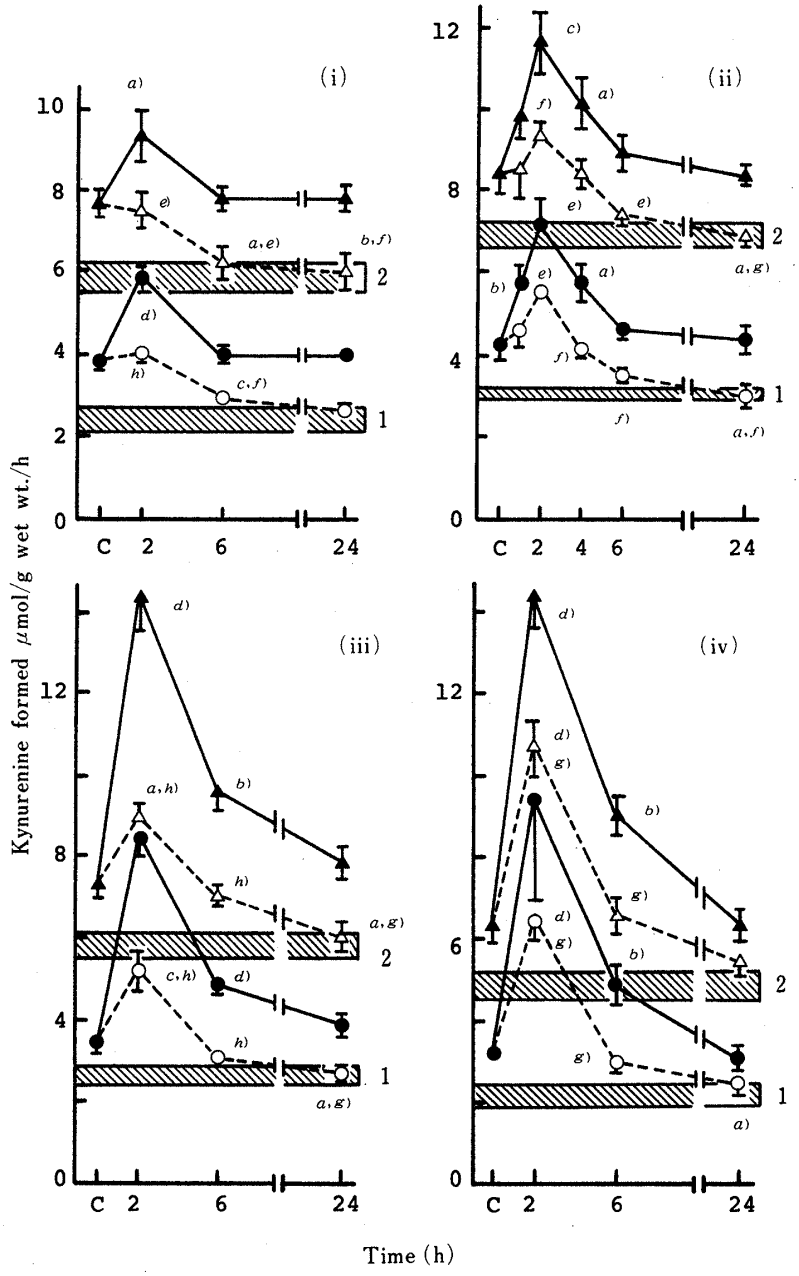

Fig. 3. Effects of Tryptophan Alone and in Combination with 3-PA on Rat Liver TP Activity

Rats were treated as described in the legend to Fig. 2. The doses of tryptophan used were as follows: i, 25 mg/kg; ii, 50 mg/kg; iii, 100 mg/kg; iv, 200 mg/kg. The enzyme activity was determined as described in Materials and Methods. Points represent the means ± S.E. (6 animals per group). ●—●, tryptophan, holoenzyme; ▲—▲, tryptophan, total enzyme; ○---○, tryptophan + 3-PA, holoenzyme; △---△, tryptophan + 3-PA, total enzyme. Shaded area 1, 3-PA, holoenzyme (the means ± S.E., 6 rats); shaded area 2, 3-PA, total enzyme (the means ± S.E., 6 rats). Significance of differences from controls: *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$. Significance of differences from tryptophan group: *e*) $p<0.05$, *f*) $p<0.02$, *g*) $p<0.01$, *h*) $p<0.001$.

The effect of a 50 mg/kg dose of tryptophan on liver TP activity is shown in Fig. 3-ii. A 37% increase in the holoenzyme activity occurred at 1 h, followed by maximum enhancement (71%) 1 h later. The enzyme activity then fell to the control level at 6 h. A similar pattern was seen in the total enzyme activity. These increases were significantly inhibited by 3-PA. At 24 h after the combined administration with 3-PA, TP holoenzyme and total enzyme activities

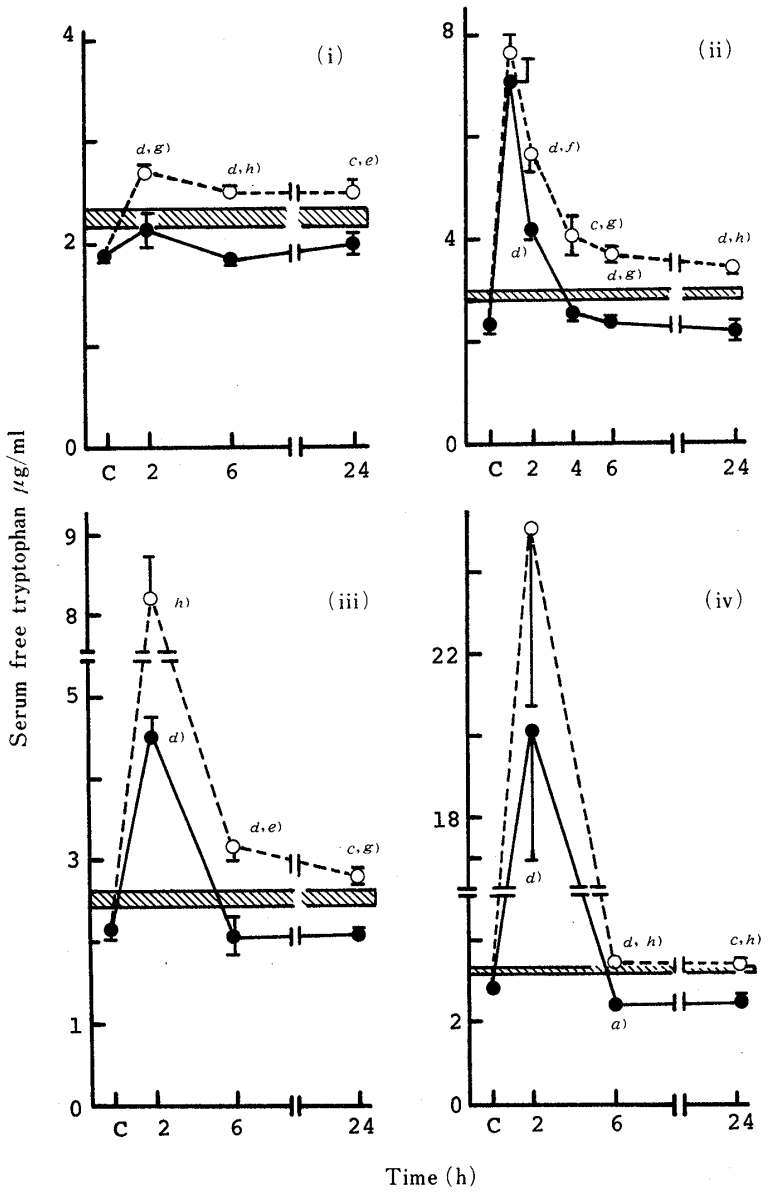

Fig. 4. Effects of Tryptophan Alone and in Combination with 3-PA on Serum Free Tryptophan Concentration in Rats

Rats were treated as described in the legend to Fig. 2. Serum free tryptophan concentration was determined as described in Materials and Methods. Points represent the means ± S.E. (6 rats). ●—●, tryptophan; ○---○, tryptophan + 3-PA; shaded area, 3-PA (the means ± S.E., 6 rats). Significance of differences from controls: *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$. Significance of differences from tryptophan group: *e*) $p<0.05$, *f*) $p<0.02$, *g*) $p<0.01$, *h*) $p<0.001$.

were not different from those in animals given 3-PA alone. Serum free tryptophan concentration was increased to 318% of the control at 1 h after tryptophan (50 mg/kg) administration, but declined to the control level at 4 h (Fig. 4-ii). The rapid decrease in serum free tryptophan was markedly prevented by 3-PA. The serum free tryptophan concentration was high even at 24 h after the combined administration.

3-PA significantly reduced the tryptophan (100 mg/kg)-induced rise in liver TP activity at all the times examined (Fig. 3-iii). At 24 h after the combined administration, the enzyme activity was at the same level as that in rats given 3-PA alone. Figure 4-iii shows that serum free tryptophan was increased to 216% of the control at 2 h after single administration of

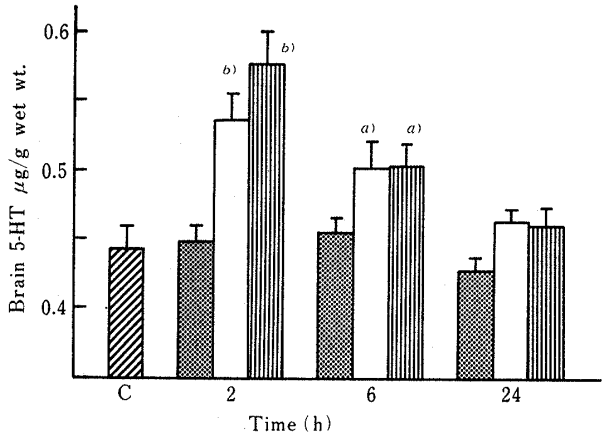

Fig. 5. Effects of Tryptophan Alone and in Combination with Allopurinol (ALLO) on Rat Brain 5-HT Concentration

Rats were given a subcutaneous injection of saline or ALLO (20 mg/kg) simultaneously with tryptophan (50 mg/kg). Control rats were injected with saline alone. Brain 5-HT concentration was determined at various times after the administration. Vertical bars represent the means ± S.E. (5 rats per group). ▨, control; □, tryptophan; ▤, tryptophan + ALLO; ⊡, ALLO. Significance of differences from control: *a*) $p<0.05$, *b*) $p<0.01$.

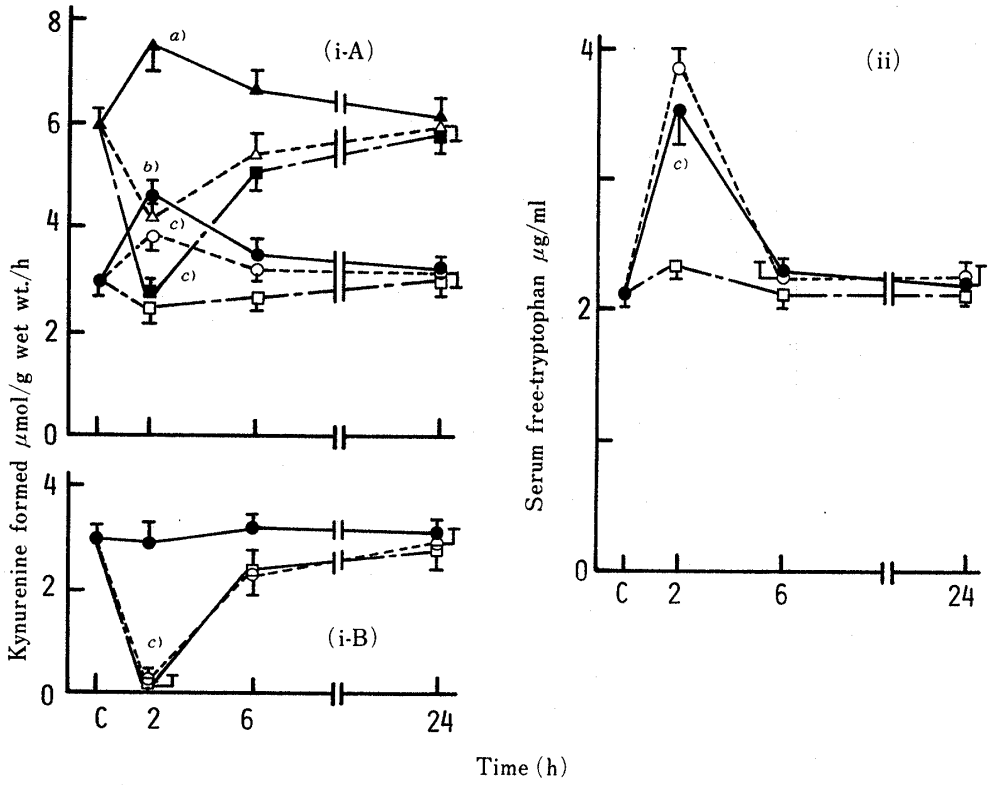

Fig. 6. Effects of Tryptophan Alone and in Combination with ALLO on Liver TP Activity (i) and Serum Free Tryptophan Concentration (ii) in Rats

Rats were treated as described in the legend to Fig. 5. TP activity and serum tryptophan concentration were determined at the indicated times after the administration. Points represent the means ± S.E. (5 rats per group).

(i-A): ●—●, tryptophan, holoenzyme; ▲—▲, tryptophan, total enzyme; □---□, ALLO, holoenzyme; ■---■, ALLO, total enzyme; ○---○, tryptophan + ALLO, holoenzyme; △---△, tryptophan + ALLO, total enzyme.

(i-B): ●—●, tryptophan, apoenzyme; □---□, ALLO, apoenzyme; ○---○, tryptophan + ALLO, apoenzyme.

(ii): ●—●, tryptophan; □---□, ALLO; ○---○, tryptophan + ALLO.

Significance of differences from controls: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

tryptophan (100 mg/kg), but rapidly declined to the control level (within 6 h). In contrast, the serum free tryptophan concentration was increased to 146 and 131% of the control at 6 and 24 h, respectively, after the combined administration with 3-PA.

The tryptophan (200 mg/kg)-induced rise in liver TP activity was also prevented by 3-PA

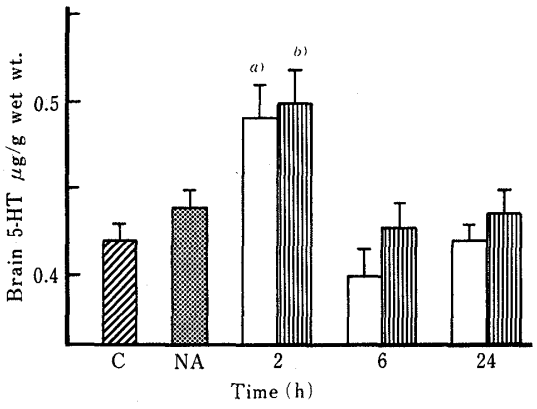

Fig. 7. Effects of Tryptophan Alone and in Combination with Nicotinamide (NA) on Rat Brain 5-HT Concentration

NA was administered to fed rats in drinking water (1 g/l) for 7 d. The control and NA-treated rats received a subcutaneous injection of either saline or tryptophan (50 mg/kg). Brain 5-HT was determined at various times after the injection. Vertical bars represent the means ± S.E. (5 rats per group). ▨, control; □, tryptophan; ▤, tryptophan + NA; ⊞, NA. Significance of differences from control: *a*) $p < 0.02$, *b*) $p < 0.01$.

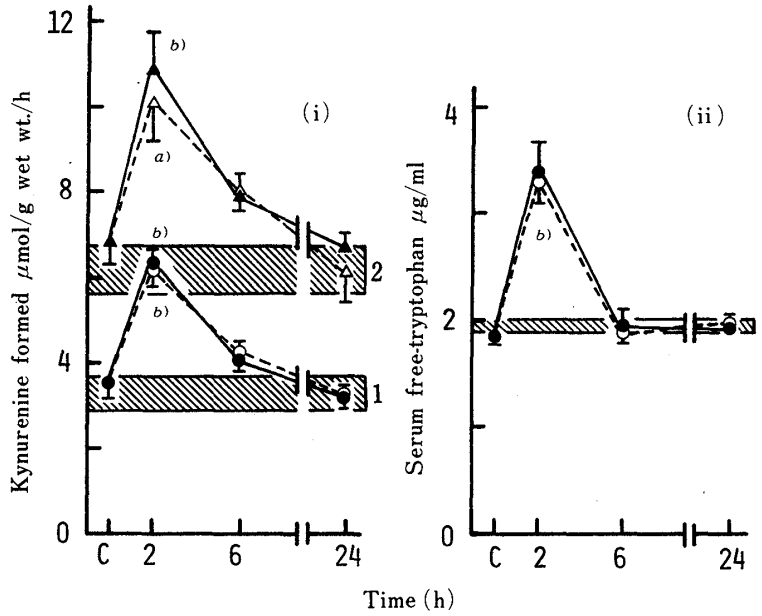

Fig. 8. Effects of Tryptophan Alone and in Combination with NA on Liver TP Activity (i) and Serum Free Tryptophan Concentration (ii) in Rats

Rats were treated as described in the legend to Fig. 7. TP activity and serum tryptophan concentration were determined at various times after the administration. Points represent the means ± S.E. (5 rats per group).

(i): ●—●, tryptophan, holoenzyme; ▲—▲, tryptophan, total enzyme; ○---○, tryptophan + NA, holoenzyme; △---△, tryptophan + NA, total enzyme; shaded area 1, NA, holoenzyme; shaded area 2, NA, total enzyme (means ± S.E., 5 rats).

(ii): ●—●, tryptophan; ○---○, tryptophan + NA; shaded area, NA (means ± S.E., 5 rats).

Significance of differences from controls: *a*) $p < 0.01$, *b*) $p < 0.001$.

(Fig. 3-iv). Figure 4-iv shows that the serum free tryptophan concentration was decreased to 87% of the control at 6 h after the administration of 200 mg/kg of tryptophan, despite the high dose. In contrast, the serum free tryptophan was increased to 120% of the control at 6 and 24 h after the combined administration with 3-PA.

Effects on Combined Administration of Tryptophan with Allopurinol on Brain 5-HT Concentration, Liver TP Activity and Serum Free Tryptophan Concentration

a) Effects on Brain 5-HT Concentration——As shown in Fig. 5, brain 5-HT concentration was slightly increased at 2 h after the combined administration of tryptophan with allopurinol, but not significantly as compared with that in rats injected with tryptophan alone. In contrast with the effect of 3-PA, a single administration of allopurinol did not increase the

brain 5-HT concentration.

b) Effects on Liver TP Activity and Serum Free Tryptophan Concentration——Liver TP apoenzyme and total enzyme activities were markedly inhibited by a single administration of allopurinol, whereas the holoenzyme activity was not affected (Fig. 6-i). At 2 h after the combined administration of tryptophan with allopurinol, the total enzyme activity was remarkably decreased, but it gradually returned to the same level as that in rats injected with tryptophan alone. The holoenzyme activity induced by tryptophan was slightly decreased by allopurinol, but not significantly.

Serum free tryptophan concentration was not increased by a single administration of allopurinol (Fig. 6-ii). The combined administration of tryptophan with allopurinol did not produce any further increase in the serum tryptophan concentration as compared with that found in animals injected with tryptophan alone.

Effects of Combined Administration of Tryptophan with Nicotinamide on Brain 5-HT Concentration, Liver TP Activity and Serum Free Tryptophan Concentration

a) Effects on Brain 5-HT Concentration——Figure 7 shows that prior administration of nicotinamide did not affect the change in brain 5-HT concentration due to tryptophan administration. A single administration of nicotinamide also did not increase the concentration of brain 5-HT.

b) Effects on Liver TP Activity and Serum Free Tryptophan Concentration——The increase in liver TP activity induced by tryptophan was not inhibited by pretreatment with nicotinamide (Fig. 8-i). The total enzyme activity was slightly decreased by a single administration of nicotinamide, but not significantly.

Figure 8-ii shows that serum free tryptophan concentration was hardly affected by the combined administration of tryptophan with nicotinamide. No significant change in serum free tryptophan concentration was found in rats given nicotinamide alone.

Although the data are not shown here, liver, kidney, spleen and small intestine 5-HT concentrations were not significantly altered by the combined administration of tryptophan with 3-PA, allopurinol or nicotinamide, as compared with those in control animals.

Discussion

Tryptophan is mainly metabolized by the kynurenine pathway, which is initiated by liver TP, an enzyme which is induced by its substrate, tryptophan. As shown in Fig. 1, the induction of liver TP by tryptophan was nearly proportional to the dose given, whereas the increase in brain 5-HT was not dose-related. The administration of 200 mg/kg of tryptophan resulted in the greatest increase in liver TP activity and the greatest decrease in brain 5-HT concentration at 6 h, as compared with those in control rats (Fig. 1-ii). These findings suggest that a high dose of tryptophan may be self-defeating because the TP induction diverts a large amount of tryptophan to the kynurenine pathway and thus the amount available for 5-HT synthesis in the brain is less than at lower doses of tryptophan. Young and Sourkes[5] also suggested that higher doses of tryptophan are ineffective for the treatment of depression. On the other hand, we showed (Fig. 1) that a 25 mg/kg dose of tryptophan did not increase the brain 5-HT even at 2 h after administration, and that only 50 mg/kg of tryptophan resulted in a significant increase in brain 5-HT at 6 h. Therefore, a major problem in administering tryptophan is the selection of the optimum dose to be given.

The tryptophan-induced rise in liver TP activity was apparently prevented by 3-PA (Fig. 3). At the same time, the combined administration of tryptophan with 3-PA caused significant increases in brain 5-HT and serum free tryptophan even at 24 h after the administration (Figs. 2 and 4). Namely, brain 5-HT and serum free tryptophan concentrations were increased more

markedly and maintained at high levels by the combined administration of tryptophan with 3-PA than by the administration of tryptophan alone. These findings suggest that the inhibition by 3-PA of the tryptophan-induced rise in liver TP activity may be related to the increase in serum free tryptophan and the change in brain 5-HT concentration may be related to the concentration of free tryptophan in the serum. It appears that the problem in administering tryptophan described above can be solved by the use of combined administration with 3-PA.

Allopurinol did not increase the brain 5-HT and serum free tryptophan concentrations in control or tryptophan-treated rats (Figs. 5 and 6). These findings indicate that allopurinol does not inhibit the catabolism of tryptophan *in vivo*. However, liver TP total enzyme and apoenzyme activities in control or tryptophan-treated rats were significantly decreased at 2 h after the injection of allopurinol (Fig. 6). Generally, the total enzyme is assayed *in vitro* with addition of exogeneous heme to activate the apoenzyme,[12] and allopurinol inhibits TP by preventing the conjugation of the apoenzyme with heme.[16] However, Joseph *et al.*[17] reported that allopurinol did not alter the metabolism of tryptophan as indicated by labelled carbon dioxide production and kynurenine concentration in plasma. They suggested that there was enough allopurinol in the liver homogenate of an allopurinol-treated rat to inhibit the conjugation of apoenzyme with exogeneous heme, and that the addition of heme to the apoenzyme *in vivo* was insensitive to allopurinol. The present results support their suggestion.

Cho-Chung and Pitot[18] reported that the activity of purified TP is allosterically inhibited by both NADPH and NADH. Badawy and Evans[10] suggested that the inhibition of TP by chronic administration of nicotinamide (0.01—5 g/l of drinking water) is mediated by both NADPH and NADH, and that this inhibition is specific to the apoenzyme. However, liver TP activity in control or tryptophan-treated rats was not significantly decreased by chronic administration of nicotinamide at the dose examined (Fig. 8-i). Brain 5-HT and serum free tryptophan concentrations were also not affected by nicotinamide administration (Figs. 7 and 8). Green *et al.*[19] also reported that chronic administration of allopurinol or nicotinamide did not affect the metabolism of an oral tryptophan load in humans.

The present results show that a TP inhibitor, 3-PA, increases the rise in serum free tryptophan concentration and prolongs the period of elevated concentration of brain 5-HT in rats injected with tryptophan. Human liver TP appears to resemble the rat enzyme in possessing both holoenzyme and apoenzyme forms in roughly equal proportions.[20] Therefore, the combined administration of tryptophan with 3-PA may be capable of producing changes in some patients with neuropsychiatric disorders. However, the combined administration of tryptophan with allopurinol or nicotinamide would not be suitable for this purpose.

References

1) P. A. Friedman, A. H. Kappelman and S. Kaufman, *J. Biol. Chem.*, **247**, 4165 (1972).
2) *a*) A. T. B. Moir and D. Eccleston, *J. Neurochem.*, **15**, 1093 (1968); *b*) D. G. Grahame-Smith, *ibid.*, **18**, 1053 (1971); *c*) J. D. Fernstrom and R. J. Wurtman, *Science*, **173**, 149 (1971).
3) *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).
4) *a*) A. Coppen, D. M. Shaw, B. Herzberg and R. Maggs, *Lancet*, **ii**, 1178 (1967); *b*) D. L. Murphy, M. Baker, F. K. Goodwin, H. Miller, J. Kotin and W. E. Bunney, *Psychopharmacology*, **34**, 11 (1974); *c*) K. Jensen, K. Fruensgaard, U. G. Ahlfors, T. A. Pihkanan, S. Toumikoski, E. Ose, S. J. Dencker, D. Lindberg and A. Nagy, *Lancet*, **ii**, 920 (1975).
5) S. N. Young and T. L. Sourkes, *Adv. Neurochem.*, **2**, 133 (1977).
6) W. E. Knox and V. H. Auerbach, *J. Biol. Chem.*, **214**, 307 (1955).
7) H. Shimeno, Y. Fukumoto, A. Toda and A. Nagamatsu, *Chem. Pharm. Bull.*, **29**, 2940 (1981).
8) *a*) B. Shopsin, *Neuropsychobiology*, **4**, 188 (1978); *b*) D. A. McSweeney, *Lancet*, **ii**, 510 (1975).
9) C. Nieman, R. N. Lewis and J. T. Hays, *J. Am. Chem. Soc.*, **64**, 1678 (1942).

10) A. A.-B. Badawy and M. Evans, *Biochem. J.*, **156**, 381 (1976).
11) J. Barchas, E. Erdelyi and P. Angwin, *Anal. Biochem.*, **50**, 1 (1972).
12) H. Kewitz and H. Wagner, *Arzneim.-Forsch.*, **15**, 1 (1965).
13) W. D. Denkla and H. K. Dewey, *J. Lab. Clin. Med.*, **69**, 160 (1967).
14) D. L. Bloxam and W. H. Warren, *Anal. Biochem.*, **60**, 621 (1974).
15) R. A. Fisher and F. Yates, "Statistical Tables for Biological, Agricultural, and Medical Research," 3rd ed., Oliver and Boyd Ltd., Edinburgh, London, 1949, p. 30.
16) A. A.-B. Badawy and M. Evans, *Biochem. J.*, **133**, 585 (1973).
17) M. H. Joseph, S. N. Young and G. Curzon, *Biochem. Pharmacol.*, **25**, 2599 (1976).
18) Y. S. Cho-Chung and H. C. Pitot, *J. Biol. Chem.*, **242**, 1192 (1967).
19) A. R. Green, J. K. Aronson, G. Curzon and H. F. Woods, *Br. J. Clin. Pharmacol.*, **10**, 611 (1980).
20) K. Altman and O. Greengard, *J. Clin. Invest.*, **45**, 1527 (1966).