J. Neural Transmission 33, 23-43 (1972). LSD 2333

The Effect of L-Tryptophan and Some Psychotropic Drugs on the Formation of 5-Hydroxytryptophan in the Mouse Brain in vivo

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With 4 Figures

Received February 15, 1972

Summary

L-Tryptophan and various agents known to interfere with the brain monoamines were injected intraperitoneally to mice. Subsequently, the accumulation of 5-hydroxytryptophan (5-HTP) in the brain induced by the intraperitoneal injection of the decarboxylase inhibitor Ro 4-4602 was investigated. Also the tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in the brain were measured.

L-Tryptophan approximately doubled the 5-HTP accumulation induced by Ro 4-4602, indicating that tryptophan hydroxylase is about half-saturated in the normal mouse brain. Inhibitors of tryptophan hydroxylase as well as nialamide, N,N-dimethyltryptamine and LSD-25 retarded the 5-HTP accumulation, whereas reserpine had no significant effect.

The data appear to support the view that the synthesis and turnover of 5-HT is regulated by a feed-back mechanism operating via changes in the intraneuronal 5-HT levels and/or in the activity of postsynaptic 5-HT receptors.

Introduction

In previous papers (*Carlsson* and *Lindqvist* 1970, *Bédard et al.* 1971) we have reported on the accumulation of 5-hydroxytryptophan (5-HTP) in the brains of rats and mice treated with the aromatic amino acid decarboxylase inhibitor Ro 4-4602. Evidence has been presented that the 5-HTP thus accumulating is formed locally in the brain, presumably in the 5-hydroxytryptamine (5-HT) carrying

neurons — cell bodies as well as fibre systems (*Bédard et al.* 1971). However, the 5-HTP accumulation in the brain was not significantly influenced by an acute transverse cerebral hemisection, interrupting the connection between the 5-HT cell bodies in the lower brain stem and their ascending fibre systems (*Bédard, Carlsson* and *Lindqvist* 1972). In contrast, a clearcut retardation of the 5-HTP formation similarly studied was found in the spinal cord below but not above an acute transection (unpublished data). From these observations as well as changes in cerebral and spinal 5-hydroxyindoleacetic acid (5-HIAA) occurring after axotomy in combination with various drug treatments we have concluded that the nerve impulse flow probably has a moderate stimulating influence on 5-HT synthesis in the central nervous system. The data indicated, however, that 5-HT synthesis takes place at a considerable rate even in the probable absence of an impulse flow and that 5-HT is largely deaminated intraneuronally under such conditions.

In the present paper we have investigated the influence on 5-HTP accumulation of the tryptophan level and of various agents interfering with some of the enzymes, transport mechanisms and receptors involved.

Methods

Groups of 6 or 12 white female mice (NMRI), 18—24 g, were used. Time and dose schedules for the drugs are given in the Result section. The animals were decapitated, the brains quickly dissected out and each brain immediately homogenized with ice-cold 0.4 N perchloric acid containing ascorbic acid, 0.2 mg/ml or in some experiments 0.5 mg/ml of anhydrous $Na_2S_2O_5$ as described by *Costa et al.* (1968), and EDTA (disodium ethylenediamine tetra-acetate, 2 mg/ml). 5-HTP, 5-HT and 5-HIAA were separated on a Dowex 50 W, X-4 column as outlined by *Atack* and *Magnusson* (1970) and described by *Lindqvist* (1971) except that the column was 4.0 (I.D.) × 72 mm instead of 4.2 mm (I.D.) × 60 mm in order to improve the recoveries of 5-HIAA. The 5-hydroxyindoles were assayed spectrophotofluorometrically (*Andén* and *Magnusson* 1967, *Lindqvist* 1971, see also *Bédard, Carlsson* and *Lindqvist* 1972). Tryptophan was eluted together with the 5-HTP and, in some experimental series, assayed by the method of *Hess* and *Udenfriend* (1959) and in others by its native fluorescence at pH 9.55 (*Bédard, Carlsson* and *Lindqvist* 1972). The latter procedure yielded about 2 μg per gram higher levels of tryptophan in the brain than did the norharman method. — In our first experimental series only 5-HTP and 5-HT were estimated. Later on, the analyses were extended to include also 5-HIAA and tryptophan.

The following drugs were used: DL-p-chlorophenylalanine methylester HCl (H 69/17), α-propyl-3,4-dihydroxyphenylacetamide (H 22/54), α-ethoxy-2,3-dihydroxyphenylacetamide (H 33/07) (Hässle*, Göteborg),

N,N-dimethyltryptamine (Aldrich), D-lysergic acid diethylamide tartrate (LSD-25, Sandoz*), nialamide HCl (Pfizer*, Näsbypark), reserpine (Ciba*, Vällingby), N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (Ro 4-4602, F. Hoffmann-La Roche & Co.*, Basle), L-tryptophan (Ajinomoto Co., Japan), L-phenylalanine ethylester HCl and L-tyrosine ethylester HCl (Fluka). All doses refer to free amino acid or base.

Statistics: In cases where comparison between more than two groups was possible, analysis of variance followed by t-test was used. Further details are given in the text and in legends of Figures and Tables.

Results

Fig. 1 shows the effect of various doses of Ro 4-4602 on the levels of 5-HTP, 5-HT and 5-HIAA in mouse brain. The time interval chosen was two hours, since this interval had been found to yield maximum 5-HTP levels (*Carlsson* and *Lindqvist* 1970). As shown in Fig. 1, it was not possible to reach plateau levels within the dose range employed. Toxicity prevented the use of substantially higher doses than 800 mg/kg. This dose was thus chosen for the subsequent experiments. In order to ensure highest possible degree of inhibition, the time interval chosen for the subsequent experiments

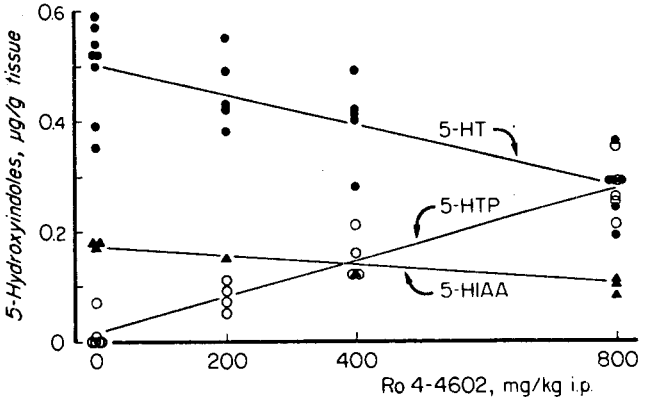

Fig. 1. 5-Hydroxyindoles in mouse brain 2 h after various i.p. doses of Ro 4-4602. Approximately linear relationships were obtained: The regression lines were calculated by the method of least squares

	5-HTP	5-HT	5-HIAA
Correlation coefficient	0.9464	—0.7882	—0.9621
Intercept	0.01557	0.5030	0.1735
Regression coefficient	0.0003241	—0.0002771	—0.00009925
n =	19	24	8

was generally 30 minutes only. In fact, in an earlier study (*Carlsson, Lindqvist* and *Waldeck* 1968) we found that the accumulation of 5-HT in brain, induced by an i.p. injection of 5-HTP 30 min before death into nialamide-pretreated mice, was completely prevented by Ro 4-4602, given in a dose of 500 mg/kg i.p. 60 min before the 5-HTP injection.

Fig. 2 and 3 show the effects of various doses of L-tryptophan on the brain levels of tryptophan and 5-hydroxyindoles in the absence and presence of Ro 4-4602, respectively. Plateau levels of all three 5-hydroxyindoles, i.e. 5-HTP (detectable only after Ro 4-4602), 5-HT and 5-HIAA, were reached at a dose of about 300 mg/kg, corresponding to a tryptophan level in the brain of about 80 μg/g (cf. *Grahame-Smith* 1971). The plateau level of 5-HTP (Fig. 3) was about 2.5 times the value obtained without L-tryptophan loading, suggesting that the tryptophan hydroxylase in the mouse brain is normally somewhat less than half-satured (cf. *Weber* and *Horita* 1965, *Green* and *Sawyer* 1966, *Moir* and *Eccleston* 1968, *Grahame-Smith* 1971, *Tagliamonte et al.* 1971). In the absence of Ro 4-4602 L-tryptophan caused a greater percentage increase in 5-HIAA (170 per cent) than in 5-HT (50 per cent) (Fig. 2). These data confirm earlier observations (*Ashcroft, Eccleston* and *Crawford* 1965) and suggest that more than half the capacity of the 5-HT stores is utilized normally.

Fig. 4a, which is based partly on data of Fig. 3, partly on data obtained from other experimental series of animals treated in similar manner, shows the effect of the tryptophan level in the brain on the accumulation of 5-HTP induced by Ro 4-4602. Fig. 4b shows the same relationship, presented as a double reciprocal plot. Extrapolation of the average of the two regression lines obtained yields an apparent K_m of about 12 μg/g. Assuming an even concentration of tryptophan in the brain and disregarding the solid matter of the tissue, an apparent K_m of about 6×10^{-5} M is calculated. *In vitro* studies on partially purified tryptophan hydroxylase from mammalian brain have yielded apparent K_m values of 2×10^{-5} M (*Ichiyama et al.* 1968) and 3×10^{-4} M (*Lovenberg, Jequier* and *Sjoerdsma* 1968), using somewhat different experimental conditions.

Table 1 shows the effect of three different tryptophan hydroxylase inhibitors, i.e. p-chlorophenylalanine (*Koe* and *Weissman* 1966) and the two dopacetamides H 22/54 and H 33/07 (*Carlsson et al.* 1963, *Carlsson* and *Corrodi* 1964). As might be expected, these inhibitors almost completely blocked the accumulation of 5-HTP induced by Ro 4-4602. The expected decrease in 5-HT levels after treatment with these inhibitors is also seen.

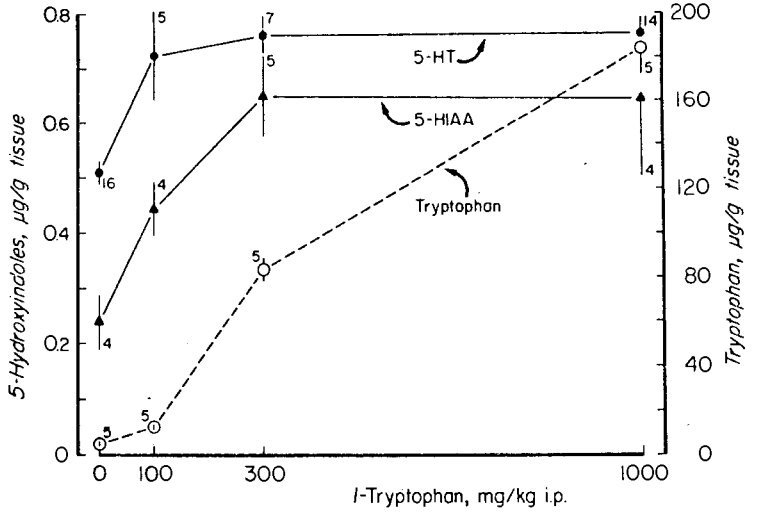

Fig. 2. Tryptophan, 5-HT and 5-HIAA levels in mouse brain 1 h after an i.p. injection of various doses of L-tryptophan.
Shown are means ± standard errors of the means. Small numbers indicate number of experiments, each comprising 4—12 mice

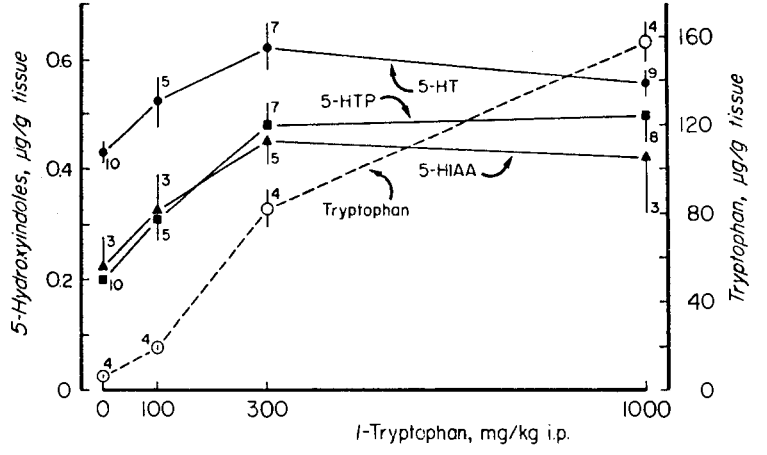

Fig. 3. Effect of various doses of L-tryptophan on the levels of various indoles in mouse brain.
Intraperitoneal injections of L-tryptophan and Ro 4-4602 (800 mg/kg) were given 60 and 30 min before death, respectively.
Shown are means ± standard errors of the means. Small numbers indicate number of experiments, each comprising 4—12 mice

Large doses of L-phenylalanine and L-tyrosine, administered as soluble ethylester hydrochlorides, caused a marked reduction in Ro 4-4602-induced 5-HTP accumulation (Table 2). Judging by the limited material available a tendency to reduced tryptophan levels was seen in the brains of the animals treated with large doses of the amino acids and Ro 4-4602. Thus competition for amino-acid carrier as well as inhibition of tryptophan hydroxylase may be involved (cf. *Grahame-Smith* 1968). The amino acids, given alone, did not seem to reduce brain 5-HT or 5-HIAA levels, although the material is too small to permit any definite conclusions.

L-phenylalanine and L-tyrosine seemed to have no marked influence on 5-HTP accumulated after pretreatment with Ro 4-4602 (Table 3). These data suggest that the above-mentioned prevention of 5-HTP accumulation (Table 2) is due to reduced formation of this amino acid.

Reserpine given in a dose depleting the brain of 5-HT (5 mg/kg i.p. about 7 h beforehand), did not interfere with the Ro 4-4602-induced 5-HTP accumulation (Table 4). When given alone, reserpine raised the 5-HIAA level in the brain ($P < 0.001$).

Nialamide (100 or 300 mg/kg i.p.), given 30 min or 2½ h before Ro 4-4602, moderately but significantly retarded the 5-HTP accumulation (Tables 5 and 6). This effect was observed both without

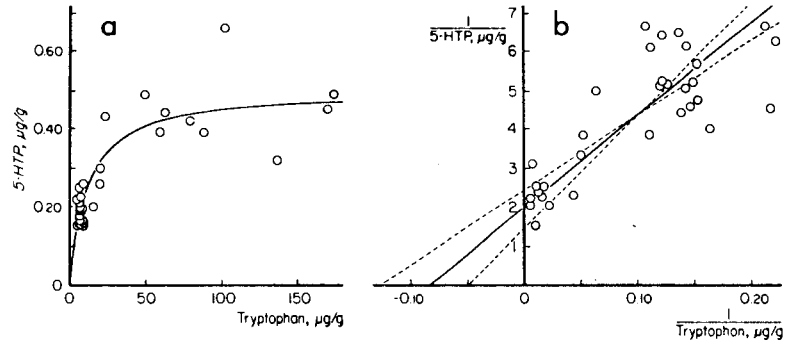

Fig. 4. a) The effect of tryptophan concentration on the rate of 5-HTP accumulation in mouse brain.

The animals were injected with Ro 4-4602, 800 mg/kg i.p. and killed 30 min later. Some of the animals received an i.p. injection of L-tryptophan (100, 300 or 1,000 mg/kg) 30 min before Ro 4-4602.

Each point represents 5-HTP and tryptophan values (µg/g) obtained from single samples of 4—12 pooled mouse brains

b) Double reciprocal plot of the same relationship. The solid line of Fig. 4 b is the average of the broken lines, representing the regression of x upon y and y upon x, respectively.

The curve of Fig. 4 a was calculated from the solid line of Fig. 4 b

Table 1. *Effect of Tryptophan Hydroxylase Inhibitors Alone and in Combination with Ro 4-4602, on the Levels of Various Indoles in Mouse Brain*

Treatment (mg/kg)	Brain levels, μg/g			
	Tryptophan	5-HTP	5-HT	5-HIAA
0	4.4 ± 0.80 (2)	0.01 ± 0.006 (3)	0.52 ± 0.018 (12)	0.20 ± 0.010 (2)
Ro 4-4602 (Ro)	8.6 ± 0.40 (2)	0.21 ± 0.012 (9)	0.40 ± 0.028 (9)	0.20 ± 0.000 (2)
PCPA (320)	6.5 ± 0.45 (2)	0.01 ± 0.005 (6)	0.34 ± 0.032 (6)	0.12 ± 0.005 (2)
PCPA (320) + Ro	7.1 ± 0.25 (2)	0.06 ± 0.013 (6)	0.29 ± 0.029 (6)	0.12 ± 0.005 (2)
H 22/54 (500)	6.6 ± 0.05 (2)	0.01 ± 0.003 (4)	0.40 ± 0.011 (4)	0.17 ± 0.020 (2)
H 22/54 (500) + Ro	7.1 ± 0.05 (2)	0.02 ± 0.011 (4)	0.31 ± 0.039 (4)	0.17 ± 0.000 (2)
H 22/54 (300)	—	0.03 ± 0.025 (2)	0.36 ± 0.020 (2)	—
H 22/54 (300) + Ro	—	0.03 ± 0.030 (2)	0.29 ± 0.010 (2)	—
H 33/07 (500)	—	0.00 ± 0.000 (2)	0.35 ± 0.005 (2)	—
H 33/07 (500) + Ro	—	0.04 ± 0.025 (2)	0.34 ± 0.015 (2)	—
H 33/07 (300)	—	0.00 ± 0.005 (2)	0.37 ± 0.040 (2)	—
H 33/07 (300) + Ro	—	0.07 ± 0.010 (2)	0.26 ± 0.015 (2)	—

p-Chlorophenylalanine (PCPA) was injected as a solution of its methylester HCl 17½ h, H 22/54 and H 33/07 1 h, and Ro 4-4602 (800 mg/kg) 30 min before death. All drugs were given intraperitoneally.

Shown are means ± standard errors of the means. Figures within brackets indicate number of experimental groups, each comprising 6—12 mice.

Analysis of variance showed all treatments to cause a significant decrease in 5-HT levels as compared to untreated controls ($p < 0.005$). The number of tryptophan and 5-HIAA analyses are too few to permit any definite conclusions.

Table 2. *Effect of L-Phenylalanine and L-Tyrosine, Alone and in Combination with Ro 4-4602, on the Levels of Various Indoles in Mouse Brain*

Treatment (g/kg)	Brain levels, μg/g			
	Tryptophan	5-HTP	5-HT	5-HIAA
0	4.0 ± 0.18 (6)	0.00 (7)	0.56 ± 0.011 (9)	0.22 ± 0.013 (7)
Ro 4-4602 (Ro)	4.5 (1)	0.18 ± 0.015 (3)	0.50 ± 0.020 (3)	—
L-Phenylalanine (2.0)	3.8 (1)	0.00 (3)	0.51 ± 0.032 (3)	0.29 (1)
L-Phenylalanine (2.0) + Ro	2.5 (1)	0.03 ± 0.024 (3)	0.38 ± 0.025 (3)	0.19 (1)
L-Phenylalanine (1.0)	3.2 (1)	0.00 (2)	0.44 ± 0.005 (2)	—
L-Phenylalanine (1.0) + Ro	2.8 (1)	0.06 ± 0.000 (2)	0.40 ± 0.100 (2)	—
L-Phenylalanine (0.3)	3.4 (1)	0.00 (2)	0.62 ± 0.090 (2)	—
L-Phenylalanine (0.3) + Ro	4.4 (1)	0.16 ± 0.010 (2)	0.44 ± 0.105 (2)	—
L-Tyrosine (2.0)	3.1 ± 0.15 (2)	0.00 (2)	0.56 ± 0.055 (2)	0.24 ± 0.030 (2)
L-Tyrosine (2.0) + Ro	2.4 ± 0.40 (2)	0.03 ± 0.025 (2)	0.37 ± 0.005 (2)	0.20 ± 0.020 (2)
L-Tyrosine (1.0)	3.3 ± 0.10 (2)	0.01 ± 0.010 (2)	0.54 ± 0.040 (2)	0.19 ± 0.040 (2)
L-Tyrosine (1.0) + Ro	4.0 ± 0.50 (2)	0.09 ± 0.020 (2)	0.39 ± 0.010 (2)	0.20 (1)

The L-amino acids (as ethylester hydrochlorides) were injected 1½ h, and Ro 4-4602 (800 mg/kg) 30 min before death. All drugs were given interperitoneally.

For further explanation see Table 1.

Table 3. *Failure of L-Phenylalanine and L-Tyrosine to Deplete the Mouse Brain of 5-HTP Accumulated after Pretreatment with Ro 4-4602*

Treatment	Brain levels, μg/g				
	Tryptophan	5-HTP	5-HT	5-HIAA	No. of exp.
Ro 4-4602 (Ro) 2 h	5.1 ± 1.19	0.26 ± 0.017	0.39 ± 0.040	0.13 ± 0.018	3
Ro, 2 h + L-Phenylalanine 30 min	3.1	0.32	0.38	0.11	1
Ro, 2 h + L-Tyrosine 30 min	2.7 ± 0.10	0.21 ± 0.020	0.43 ± 0.135	0.12 ± 0.010	2
Ro, 1 h	5.7	0.26	0.31	0.16	1
Ro, 1 h + L-Tyrosine 30 min	2.0	0.18	0.45	0.18	1

Ro 4-4602 (800 mg/kg), L-Phenylalanine (2.0 g/kg), and L-Tyrosine (2.0 g/kg) were administered intraperitoneally (for details, see Table 2). Intervals refer to time before death.

For further explanation see Table 1.

Table 4. *Effect of Reserpine, Alone or in Combination with Ro 4-4602 on the Levels of Various Indoles in Mouse Brain*

Treatment			Brain levels, μg/g			
Reserpine	Ro 4-4602					
mg/kg i.p.	mg/kg i.p.	Time before death	Try	5-HTP	5-HT	5-HIAA
—	—	—	5.2 ± 0.18 (4)	0.00 (4)	0.55 ± 0.027 (5)	0.18 ± 0.017 (5)
5	—	—	4.7 ± 0.34 (5)	0.00 (4)	0.03 ± 0.011 (6)	0.33 ± 0.016 (6)
—	800	30 min	—	0.20 ± 0.015 (4)	0.36 ± 0.046 (4)	—
5	800	30 min	—	0.18 ± 0.003 (3)	0.06 ± 0.018 (3)	—
—	800	2 h	5.9 ± 0.17 (4)	0.25 ± 0.011 (8)	0.36 ± 0.019 (8)	0.12 ± 0.011 (5)
5	800	2 h	5.9 ± 0.33 (4)	0.28 ± 0.022 (8)	0.01 ± 0.005 (8)	0.12 ± 0.007 (5)

Reserpine was injected 7—8 h before death. The animals were kept at an ambient temperature of +30 °C 4 h before death.

For further explanation see Table 1.

and with L-tryptophan loading causing a high degree of saturation of the tryptophan hydroxylase. Nialamide caused the expected increase in 5-HT and decrease in 5-HIAA levels.

The effect of N,N-dimethyltryptamine was studied both without (Table 7) and with (Table 8) L-tryptophan pretreatment. In both cases this hallucinogen, when given in a relatively large dose (50 mg/kg i.p.) moderately retarded the Ro 4-4602-induced 5-HTP accumulation. Analysis of variance on the data obtained with this dose, with and without L-tryptophan loading, revealed that the effect was significant ($P < 0.01$: p×q factorial experiment, *Winer*

Table 5. *Effect of nialamide and Ro 4-4602 on the Levels of 5-HTP and 5-HT in Mouse Brain*

Treatment	Brain levels, μg/g	
	5-HTP	5-HT
0	0.00 (10)	0.52 ± 0.021 (10)
Nialamide	0.00 ± 0.003 (4)	1.48 ± 0.107 (4)
Ro 4-4602	0.19 ± 0.025 (4)	0.42 ± 0.033 (4)
Nialamide + Ro 4-4602	0.15 ± 0.018 (4)[1]	1.20 ± 0.078 (4)

Nialamide (300 mg/kg i.p.) was injected 3 h and Ro 4-4602 (800 mg/kg i.p.) 30 min before death.

For further explanation see Table 1.

[1] Significantly different from Ro 4-4602 ($P < 0.01$, process of pairing).

1962). N,N-Dimethyltryptamine also caused a significant increase in 5-HT levels ($P < 0.01$: analysis of variance as above), whereas the 5-HIAA levels were reduced ($P < 0.001$: one-way analysis of variance on data after L-tryptophan loading).

LSD-25 in doses of 1 to 6 mg/kg caused a moderate but significant decrease in the Ro 4-4602-induced 5-HTP accumulation (Table 9). The effect was observed both with and without L-tryptophan loading. LSD-25 also caused a significant, though apparently short-lasting increase in 5-HT and decrease in 5-HIAA levels. However, neither of these effects were detected in the group pretreated with L-tryptophan.

The ability of Ro 4-4602 to lower 5-HT levels was detected both in the absence and in the presence of LSD-25 but seemed to be somewhat less pronounced in LSD-25-treated animals.

Table 6. *Effect of Nialamide and Ro 4-4602 on the Levels of Various Indoles in Mouse Brain after L-Tryptophan Loading*

Treatment	Brain levels, μg/g			
	Try	5-HTP	5-HT[3]	5-HIAA
1) 0	7.8 ± 0.78 (5)	0.00 (5)	0.57 ± 0.026 (5)	0.21 ± 0.011 (5)
2) L-Tryptophan (Try)	49.2 ± 6.91 (6)[1]	0.00 (6)	0.77 ± 0.048 (7)	0.63 ± 0.034 (7)[4]
3) Try + Ro 4-4602	67.8 ± 6.20 (6)	0.46 ± 0.021 (6)	0.56 ± 0.023 (7)	0.44 ± 0.030 (7)[4]
4) Try + Nialamide (N)	81.1 ± 6.05 (6)	0.00 (6)	1.41 ± 0.067 (7)	0.28 ± 0.018 (7)
5) Try + N + Ro 4-4602	90.8 ± 6.09 (6)	0.30 ± 0.028 (6)[2]	1.00 ± 0.034 (7)	0.28 ± 0.021 (7)

L-Tryptophan (300 mg/kg i.p.) was injected 1 h 15 min, nialamide (100 mg/kg i.p.) 1 h, and Ro 4-4602 (800 mg/kg i.p.) 30 min before death.

For further explanation see Table 1.

[1] Significantly different from 3) ($p = 0.05$), 1) and 3) to 5) ($P < 0.005$).

[2] Significantly different from 3) ($P < 0.001$).

[3] All differences significant ($P < 0.005$) except 1)—3).

[4] Significantly different from all the other groups ($P < 0.001$).

Table 7. *Effect of N,N-Dimethyltryptamine and Ro 4-4602 on the Levels of 5-HTP and 5-HT in Mouse Brain*

Treatment			Brain levels, μg/g		No. of experiments
N,N-Dimethyltryptamine		Ro 4-4602			
mg/kg i.p.	Time before death	mg/kg i.p.	5-HTP	5-HT	
—	—	—	0.00	0.52 ± 0.021	10
—	—	800	0.24 ± 0.009	0.40 ± 0.031	8
25	60 min	—	0.03 ± 0.008	0.66 ± 0.087	4
25	60 min	800	0.19 ± 0.020	0.47 ± 0.075	4
25	40 min	—	0.01	0.55	1
25	40 min	800	0.22	0.52	1
50	40 min	—	0.01 ± 0.003	0.79 ± 0.013	4
50	40 min	800	0.15 ± 0.000	0.57 ± 0.033	4

Ro 4-4602 was injected 30 min before death. Shown are means ± standard errors of the means. Each experiment comprises 12 mice.

Table 8. *Effect of N,N-Dimethyltryptamine (DMT) and Ro 4-4602 on the Levels of Various Indoles in Mouse Brain after L-Tryptophan Loading*

Treatment	Brain levels, μg/g				No. of experiments
	Try	5-HTP	5-HT	5-HIAA	
0	5.1 ± 0.47	0	0.48 ± 0.072	0.25 ± 0.007	3
L-Tryptophan (Try)	57.9 ± 10.31	0	0.64 ± 0.091	0.62 ± 0.022	3
Try + Ro 4-4602 (Ro)	48.2 ± 2.89	0.48 ± 0.045	0.45 ± 0.061	0.52 ± 0.029	3
Try + DMT	54.9 ± 9.82	0	1.07 ± 0.177	0.43 ± 0.022	3
Try + DMT + Ro	50.2 ± 6.98	0.30 ± 0.038	0.81 ± 0.061	0.30 ± 0.033	3

L-Tryptophan (300 mg/kg i.p.) was injected 1 h 15 min, DMT (50 mg/kg i.p.) 40 min, and Ro 4-4602 (800 mg/kg i.p.) 30 min before death.

Shown are means ± standard errors of the means. Each experiment comprises 6 mice.

Discussion

The present investigation should be regarded as a preliminary survey of the effect of various agents on the accumulation of 5-HTP induced by decarboxylase inhibition. Many of the observations made hardly permit any definite conclusions but rather the formulation of questions to be investigated in more detailed studies.

Previous investigations aiming to analyze the first step in the biosynthesis of 5-HT have generally been based on indirect evidence, e.g. on measurements of 5-HT and 5-HIAA levels, using conventional or tracer techniques, partly in combination with various drug treatments. These investigations have yielded much useful information, but the interpretation has often proved difficult. The present approach where the accumulation of 5-HTP is measured after decarboxylase inhibition, is more direct and thus avoids some of the difficulties encountered earlier. The data shown in the present study suggest that the procedure employed gives a reliable measure of the rate of hydroxylation of tryptophan.

The 5-HTP accumulating after decarboxylase inhibition seems to disappear slowly. This conclusion can be tentatively drawn from the failure of L-phenylalanine and L-tyrosine (which markedly inhibit 5-HTP accumulation), to deplete the brain of 5-HTP accumulated after pretreatment with the decarboxylase inhibitor. Thus the rate of 5-HTP accumulation appears to be mainly determined by its rate of formation.

It must be admitted, however, that the present approach is probably not deviod of pitfalls. For example, the possibility cannot be excluded that the accumulation of 5-HTP or the partial depletion of 5-HT induced by decarboxylase inhibition may influence feed-back mechanisms acting on the tryptophan hydroxylase or the transport of its substrate into the neuron. Moreover, the decarboxylase inhibitor, which had to be used in a large dose, may influence the rate of tryptophan hydroxylation through unspecific mechanisms. Some caution thus has to be exercised in interpreting the data obtained.

The observations made after various doses of L-tryptophan (Fig. 1 and 2) indicated that maximum levels of 5-HTP (after Ro 4-4602), 5-HT and 5-HIAA with or without Ro 4-4602) were reached at the same dose (300 mg/kg) and the same concentration (about 80 μg/g) of tryptophan in the brain, which is thus apparently the concentration required for complete saturation of the tryptophan hydroxylase. These observations, as well as the fairly good agreement between *in-vivo* and *in-vitro* estimates of apparent K_m values, argue in favour of the 5-HTP accumulation as a reliable indicator of tryptophan hydroxylase activity *in-vivo*.

The data obtained after administration of tryptophan hydroxylase inhibitors (Table 1) are entirely in accordance with expectation. On the other hand, the reduced 5-HTP accumulation observed after treatment with L-phenylalanine and L-tyrosine is difficult to reconcile with the lack of a clearcut effect of these amino acids in the same dosage on 5-HT and 5-HIAA levels. These data suggest an interaction between Ro 4-4602 and the amino acids. A more detailed investigation, involving e.g. estimation of amino acid levels in the brain, is necessary to clarify this problem.

The lack of effect of reserpine on the 5-HTP accumulation induced by Ro 4-4602 is unexpected, since reserpine causes an increase

Table 9 (foot notes)

Ro 4-4602 was given in a dose of 800 mg/kg i.p. 30 min before death. LSD-25, when given in a dose of 6 or 3 mg/kg i.p., was administered 60 min before death. In the remaining groups LSD-25 was given 40 min before death. In two of these groups an additional dose was given as indicated 20 min before death. L-tryptophan was given i.p. as indicated 1 h 15 min before death.

For further explanation see Table 1.

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[1] Analysis of variance of the differences between matched pairs of groups treated with Ro 4-4602 and LSD-25 + Ro 4-4602, respectively, gave the following result for the different doses of LSD-25: 6 mg/kg: $P > 0.10$, 3 mg/kg: $P < 0.001$, 2+1 mg/kg: $P > 0.10$, 1+5 mg/kg: $P = 0.01$, 1 mg/kg: $P < 0.025$, 1+0.5 mg/kg (after L-tryptophan loading): $P < 0.001$.

[2] One-way analysis of variance showed that LSD-25 alone caused a significant increase in 5-HT ($P < 0.005$), when given in the largest single dose (6 mg/kg) or in repeated dosage. In the groups treated with Ro 4-4602, LSD-25 caused a significant increase in 5-HT as compared to Ro 4-4602 alone ($P < 0.005$), except in the lower dose (3 mg/kg) at the longer interval (60 min). After L-tryptophan loading LSD-25 caused no significant effect on 5-HT ($P > 0.05$). When both L-tryptophan and Ro 4-4602 were given, LSD-25 caused a significant increase in 5-HT ($P < 0.025$), as compared to L-tryptophan + Ro 4-4602.

Ro 4-4602, when compared to the corresponding group not treated with this agent, caused a significant decrease in 5-HT ($P < 0.001$ in most cases) except when given in combination with LSD-25 in the two lower single doses (3 and 1 mg/kg).

[3] One-way analysis of variance showed that LSD-25 alone caused a significant decrease in 5-HIAA ($P < 0.01$), when given in doses of 1 or 1+0.5 mg/kg. In the groups treated with Ro 4-4602, the same effect of LSD-25 was observed in all groups ($P < 0.01$), except in the L-tryptophan pretreated group. Ro 4-4602 in all cases caused a significant decrease ($P < 0.025$ in most cases).

Table 9. *Effect of LSD-25, Alone and in Combination with Ro 4-4602, on the Levels of Various Indoles in Mouse Brain*

Treatment (mg/kg)	Brain levels, μg/g				No. of experiments
	Try	5-HTP[1]	5-HT[2]	5-HIAA[3]	
0	—	0.00 ± 0.000	0.60 ± 0.024	—	6
Ro 4-4602 (Ro)	—	0.21 ± 0.016	0.42 ± 0.032	—	6
LSD-25 (6)	—	0.00 ± 0.003	0.81 ± 0.068	—	3
LSD-25 (6) + Ro	—	0.16 ± 0.012	0.62 ± 0.046	—	3
LSD-25 (3)	—	0.02 ± 0.012	0.56 ± 0.055	—	3
LSD-25 (3) + Ro	—	0.15 ± 0.020	0.47 ± 0.033	—	3
0	6.0 ± 0.20	0.01 ± 0.003	0.63 ± 0.013	0.25 ± 0.009	10
Ro 4-4602	7.1 ± 0.18	0.19 ± 0.007	0.46 ± 0.016	0.22 ± 0.008	10
LSD-25 (2+1)	8.3 ± 0.90	0.01 ± 0.013	0.95 ± 0.065	0.24 ± 0.020	2
LSD-25 (2+1) + Ro	8.7 ± 0.15	0.18 ± 0.027	0.67 ± 0.080	0.16 ± 0.000	2
LSD-25 (1+0.5)	6.3 ± 0.20	0.00 ± 0.000	0.75 ± 0.025	0.17 ± 0.006	5
LSD-25 (1+0.5) + Ro	7.3 ± 0.26	0.14 ± 0.008	0.63 ± 0.013	0.13 ± 0.004	5
LSD-25 (1)	5.8 ± 0.06	0.02 ± 0.007	0.68 ± 0.018	0.20 ± 0.009	3
LSD-25 (1) + Ro	7.2 ± 0.47	0.14 ± 0.028	0.59 ± 0.018	0.16 ± 0.012	3
0	5.7 ± 0.49	0.01 ± 0.002	0.60 ± 0.028	0.25 ± 0.018	4
L-Tryptophan (Try) (300)	62.8 ± 11.28	0.00 ± 0.005	0.88 ± 0.034	0.69 ± 0.031	4
Try (300) + Ro	65.4 ± 7.36	0.54 ± 0.056	0.65 ± 0.030	0.53 ± 0.036	4
Try (300) + LSD-25 (1+0.5)	61.4 ± 7.03	0.00 ± 0.005	0.96 ± 0.047	0.64 ± 0.047	4
Try + LSD-25 (1+0.5) + Ro	64.2 ± 10.57	0.45 ± 0.042	0.76 ± 0.055	0.45 ± 0.019	4

in the 5-HIAA level in the brain. This effect persists after probenecid pretreatment (*Werdinius* 1967), suggesting an increase in the rate of synthesis of 5-HT and 5-HIAA. Further work is needed to clarify this apparent discrepancy.

The monoamine oxidase inhibitor nialamide caused a moderate retardation of the 5-HTP accumulation induced by decarboxylase inhibition. This might be taken as support for the existence of a feed-back mechanism reducing the rate of tryptophan hydroxylation when the 5-HT level intraneuronally or at the postsynaptic receptor sites is increased, and *vice versa*. Needless to say, direct partial inhibition of tryptophan hydroxylase by nialamide cannot be ruled out. Preliminary data on mouse brain slices indicate, however, that nialamide does not influence the rate of tryptophan hydroxylation *in vitro* (unpublished data of this laboratory).

The hallucinogenic agents N,N-dimethyltryptamine and LSD-25 both moderately retarded the 5-HTP accumulation. The mechanisms of these effects remain to be elucidated. Preliminary data on mouse brain slices give no support for a direct inhibition of tryptophan hydroxylase (unpublished data of this laboratory). Therefore, again some kind of feed-back inhibition appears to be the most likely alternative. Like nialamide, both agents caused an increase in total 5-HT levels in the brain, which is in agreement with earlier observations (*Freedman* 1961, *Diaz, Ngai* and *Costa* 1968). The feed-back mechanism possibly involved may therefore be brought into play by changes in intraneuronal 5-HT levels. The two hallucinogenic agents may also influence a feed-back mechanism via a direct receptor activation (cf. *Andén, Corrodi, Fuxe* and *Hökfelt* 1968, *Andén, Corrodi* and *Fuxe* 1971). It should be pointed out that in the large dosage used N,N-dimethyltryptamine has probably multiple sites of attack, i.e. monoamine oxidase inhibition (unpublished data of this laboratory, see also *Pletscher, Gey* and *Burkard* 1966), blockade of 5-HT uptake (*Carlsson* 1970) and release of 5-HT (*Fuxe* and *Meek* 1971).

Not only nialamide and N,N-dimethyltryptamine but also LSD-25 caused an increased 5-HT and a decreased 5-HIAA level in the brain, in agreement with earlier observations (*Rosecrans et al.* 1967, *Aghajanian* and *Weiss* 1968, *Diaz et al.* 1968, *Tonge* and *Leonard* 1969). LSD-25 has also been found to reduce the amount of ^{14}C-5-HT recovered from the brain after administration of ^{14}C-tryptophan (*Lin et al.* 1969) and the spontaneous electrical activity of the 5-HT cell bodies in the mesencephalic raphe region (*Aghajanian et al.* 1970). All these observations might be explained on the basis of a feed-back control of 5-HT neurons, brought into play by post-

synaptic receptor activation. Evidence for the existence of such mechanisms in central monoaminergic synapses has accumulated during the last decade (see *Andén, Carlsson* and *Häggendal* 1969).

Remarkably enough the increased 5-HT and decreased 5-HIAA levels induced by LSD-25 could not be detected in animals pretreated with L-tryptophan. The explanation of this phenomenon may possibly be sought in the considerable increase in intraneuronal 5-HT synthesis and turnover induced by L-tryptophan. At this high level of metabolism already in the resting state changes in the superimposed activity-regulated 5-HT turnover may be less readily detected. However, the Ro 4-4602-induced 5-HTP accumulation was retarded by LSD-25 even after L-tryptophan loading. Further studies are needed to clarify the interaction between L-tryptophan and LSD-25.

Acknowledgements

This study has been supported by grant B72-14X-155-08A from the Swedish Medical Research Council.

For generous supplies of drugs we are indebted to the drug companies indicated by an asterisk in the text.

For valuable help with statistical problems and preparation of the figures we thank Research Engineer *Tor Magnusson*.

The technical assistance of Mrs. *Ingrid Bergh*, Miss *Arja Lahtinen* and Mrs. *Birgitta Nyström* is gratefully acknowledged.

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