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Fenfluramine-Induced Behavior Changes in Rats Prefed Serotonin-Altering Amounts of Tryptophan and Pyridoxine[1]

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LEE, N S , G C WAGNER, J R TROUT AND H FISHER *Fenfluramine-induced behavior changes in rats prefed serotonin-altering amounts of tryptophan and pyridoxine* PHARMACOL BIOCHEM BEHAV **29**(3) 565–571, 1988 —It has been well established that elevated dietary tryptophan (TRP) levels can increase brain serotonin concentrations, thereby influencing serotonergic transmission We previously examined interaction between dietary substrate (TRP 0 15 and 0 6%) and the cofactor precursor (pyridoxine HCl 3 and 3,000 mg/kg) on brain serotonin metabolism, observing significant increases in serotonin concentrations from such dietary interaction The present experiments were designed to explore possible behavioral consequences of the substrate-cofactor interaction After the IP injection of fenfluramine (FA at 5, 10, 15, and 20 mg/kg), serotonin-mediated behavior traits and the appearance of flushing were observed in rats fed experimental diets as stated above With a 5 mg/kg dose of FA, a differential dietary effect was most visible However, at higher FA levels (15 and 20 mg/kg), such dietary effects were no longer discernible The appearance of flushing was also dependent on dietary TRP intake and the dosage of FA These results indicate a clear substrate-cofactor interaction on certain serotonin-mediated behavior traits in the rat

Tryptophan Serotonin Behavior Hypothalamus Fenfluramine Vitamin B_6 Brain

RECENT research has demonstrated that manipulation of brain serotonin levels through changes in the diet affect serotonergically mediated behaviors—sleep, depression, pain sensitivity, food intake, temperature and blood pressure regulation, and aggressive behavior among others [2, 4, 9, 12, 16, 19, 28, 30]

In a previous study, we investigated the metabolic interaction of dietary tryptophan and a high intake of vitamin B_6 on hypothalamic serotonin concentrations of rats. It was demonstrated that not only dietary tryptophan, but the combination of tryptophan plus a high level of pyridoxine influenced serotonin metabolism in the rat brain [17]

Vitamin B_6, as the precursor of the coenzyme pyridoxal-5-phosphate (PLP), plays an important role in the metabolism of various compounds including the neurotransmitter serotonin Serotonin is synthesized from the dietary amino acid tryptophan by a two-step reaction, hydroxylation, followed by decarboxylation The enzyme 5-hydroxytryptophan decarboxylase requires PLP as a cofactor. Pyridoxine deficiency has been shown to cause a decrease in brain serotonin concentration [7] and the intraperitoneal injection of pyridoxine has been shown to cause an increase in tryptophan levels followed by a concomitant rise in brain serotonin [1].

High doses of vitamin B_6 have been shown to cause a neurotoxicity to sensory neurons in animals [15, 25, 26] A similar neurotoxic effect of high intakes of pyridoxine has been reported recently in several clinical cases [24,27] In both the human and animal studies, the authors did not find central nervous system toxicities which would account for their observations. However, they did not include any neurochemical measurements in their studies.

Behavioral effects from a high pyridoxine intake have been studied by Driskell and Loker [8] who noted a reduced exploration and curiosity effect in rats fed high levels of pyridoxine (350 times the National Research Council (NRC) recommended level) Bender and Totoe [1], in their study of metabolic effects from high intakes of pyridoxine also reported a similar sedative effect Both groups of researchers speculated that these sedative effects might have been due to increased cerebral serotonin resulting from high vitamin B_6 feeding

Recently, the occurrence of a stereotyped, serotonin-mediated behavioral syndrome has been described following the administration of drugs such as fenfluramine that release serotonin [14]. This syndrome consists of resting tremor, rigidity or hypertonus, reciprocal front paw treading, Straub tail, hindlimb abduction, lateral head weaving, head shaking,

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TABLE 1

ANALYSIS OF VARIANCE OF DIETARY TREATMENT, BEHAVIORAL OBSERVATIONS, FENFLURAMINE ADMINISTRATION AND THEIR INTERACTIONS p-VALUES FOR F

		Log Transformed Behavior Data							
Source of Variance	*df*	Backward Walking	Front Paw Treading	Head Weaving	Hind Limb Abduction	Straub Tail	Circling	Two Paws on wall	Grooming
TRP	1	0 67	0 77	0 66	0 056*	0 43	0 01*	0 14	0 09
B_6	1	0 12	0 73	0 05*	0 04*	0 16	0 96	0 60	0 11
TRP×B_6	1	0 10	0 56	0 08	0 14	0 69	0 81	0 02*	0 33
MINE	3	0 005*	0 017*	0 001*	0 06	0 002*	0 0006*	0 59	0 66
TRP×MINE	3	0 53	0 33	0 27	0 11	0 41	0 06	0 81	0 58
B_6×MINE	3	0 86	0 72	0 009*	0 43	0 66	0 28	0 63	0 83
TRP×B_6×MINE	3	0 58	0 99	0 90	0 79	0 91	0 74	0 48	0 64

TRP=Tryptophan (0 15%, 0 6%)
B_6=Pyridoxine HCl (7, 3,000 mg/kg)
MINE=Fenfluramine (5, 10, 15, 20 mg/kg body weight)
*=Statistically significant

hyperactivity, hyperreactivity and salivation The above syndrome is thought to be serotonergically-mediated, because injection of the serotonin precursors, 5-hydroxytryptophan (5HTP) or tryptophan produce the syndrome [14] The precursor effects are prevented by prior inhibition of serotonin synthesis mediated through tryptophan hydroxylase or 5-hydroxytryptophan decarboxylase and is unaffected by prior inhibition of catecholamine synthesis [14]

The purpose of the present study was to explore the behavioral consequences of the dietary tryptophan-pyridoxine interaction The drug fenfluramine was used to facilitate this behavioral study Fenfluramine has been reported to produce a sustained release of serotonin from storage granules with only transient or little effect on central nervous system levels of norepinephrine or dopamine [10] The short latency period, 3–5 minutes following intraperitoneal injection, indicates that the mode of action of fenfluramine is via a rapid release of serotonin from the storage granules [32] Other researchers [3, 10, 20] have used fenfluramine in studies of behavioral traits that are mediated by serotonergic pathways

METHOD

Animals

Sprague Dawley C/D male weanling rats (body weight 50–60 g, Charles River Company, Wilmington, MA) were used for all experiments They were housed individually in wire cages with a 12 hour light dark cycle (lights off at 19·00 hr) and had free access to the experimental diets and to water The animals were weighed and their food consumption recorded three times a week

After 10 days on the experimental diets, they were observed for signs of the serotonin-mediated behavior syndrome and were subsequently sacrificed by decapitation for post-fenfluramine serotonin and 5-hydroxyindoleacetic acid (5HIAA) determinations in the hypothalamus Hypothalamic tissues were immediately removed, frozen in liquid nitrogen and stored at −80°C until the time of assay, which was not more than two weeks

Diets

The isocaloric, isonitrogeneous, semi-purified experimental diets provided the minimum daily requirement of protein plus amino acids (13%) for young growing rats. The diets provided two levels of tryptophan (0.15 and 0.6%) and two levels of pyridoxine HCl (7 and 3,000 mg/kg). The lower level of tryptophan represented the reported NRC requirement level [23] for this amino acid and the higher level was chosen to be similar to the concentration found in commercial rat diets The lower level of pyridoxine represented the NRC recommended level (6 mg/kg diet) and the higher level was approximately 400 times that of the lower level The composition of the experimental diets has been previously reported [17]

Behavioral Observations

After 10 days on the experimental diets, different groups of rats were weighed and injected intraperitoneally with varying amounts of fenfluramine 0, (saline) 5, 10, 15, or 20 mg/kg body weight The rats were then individually placed in Plexiglas cages (44×24×20 cm) After 10 minutes, they were observed for one hour for any serotonin-mediated behaviors All behavioral observations were carried out between 9 am and 12 noon The observer was unaware of the diet treatment and the dosage of fenfluramine Four animals, in separate cages, were observed simultaneously All movements believed to be associated with serotonin were counted and recorded These movements included backward walking, head weaving, hind limb abduction, front paw treading, circling, two paws on the wall, Straub tail, and grooming A number of animals exhibited extreme redness of their noses and limbs, and this phenomenon was also recorded Animals were considered to be flushed when their noses, tails and paws turned deep pink, which occurred usually within twenty minutes after fenfluramine injection The appearance of flushing was recorded in an all or none fashion (+ or −) After completing the behavioral observations, the animals were sacrificed by decapitation, and the hypothalamus removed quickly for post-fenfluramine serotonin and 5HIAA analyses

TABLE 2A

EFFECT OF FENFLURAMINE DOSE ON FREQUENCY OF BEHAVIORAL MEASUREMENT

Fenfluramine (mg/kg)	Backward Walking	Front Paw Treading	Head Weaving	Hind Limb Abduction	Straub Tail	Circling	Two Paws on Wall	Grooming
5	3 4*	9 0*	11 3*	1 0*	1 4*	7 5*	7 9*	2 8*
10	9 5†	21 4†	40 7†	1 3*	3 6*†	16 4†	7 5*	3 7*
15	11 3†	23 0†	63 6†‡	1 6*†	8 8†	20 4†	7 5*	2 2*
20	16 0†	24 7†	96 7‡	3 1†	9 7†	22 4†	5 0*	2 5*

N=16

Within each column, values with different superscripts are statistically significantly different from each other ($p<0$ 05, Duncan's Multiple Range Test)

TABLE 2B

EFFECT OF TRYPTOPHAN LEVEL ON FREQUENCY OF BEHAVIORAL MEASUREMENT

Tryptophan (% of diet)	Backward Walking	Front Paw Treading	Head Weaving	Hind Limb Abduction	Straub Tail	Circling	Two Paws on Wall	Grooming
0 15	8 8*	17 6*	38 8*	1 2*	4 1*	12 2*	5 8*	2 1*
0 5	9 5*	19 0*	42 7*	2 2†	5 6*	19 4†	8 3*	3 6*

N=32

Within each column values with different superscripts are statistically significantly different from each other ($p<0$ 05, Duncan's Multiple Range Test, except for hind limb abduction where $p=0$ 056)

TABLE 2C

EFFECT OF PYRIDOXINE LEVEL ON FREQUENCY OF BEHAVIORAL MEASUREMENT

Pyridoxine HCl (mg/kg diet)	Backward Walking	Front Paw Treading	Head Weaving	Hind Limb Abduction	Straub Tail	Circling	Two Paws on Wall	Grooming
7	6 9*	17 6*	32 1*	1 2*	3 7*	15 6*	6 4*	3 5*
3,000	11 0*	19 0*	51 5*	2 2†	6 2*	15 6*	7 3*	2 1*

N=32

Within each column, values with different superscripts are statistically significantly different from each other ($p<0$ 05, Duncan s Multiple Range Test)

Statistical Analysis

The behavioral frequency measurements are presented in Tables 2A–2D as the differences between values observed with 5, 10, 15, and 20 mg/kg fenfluramine and those obtained with the saline controls (0 fenfluramine) The serotonin-mediated behavior results as well as the post-fenfluramine neurochemical data were analyzed by analysis of variance (ANOVA) To improve the normality of the values being compared, the frequency of the measurements were log transformed by the equation $\log_{10}(X + 1)$ The log transformed behavioral data and the post-fenfluramine neurochemical data were analyzed for main effects of fenfluramine dose, dietary tryptophan and dietary pyridoxine in a 4×2×2 ANOVA using SAS (SAS Institute, Inc , Box 8000, Cary, NC). For the factors found to give statistically significant responses, Duncan's Multiple Range Test was used as a multiple comparison procedure to identify significant differences among individual means of log transformed data The results displayed in the tables are geometric means obtained by reversing the transformation of the means of the log tranformed data The data on the occurrence of flushing were analyzed by the Chi-square contingency table analysis Factors examined were dietary tryptophan, vitamin B_6 and fenfluramine dose

RESULTS

The amount of fenfluramine injected significantly influenced most of the behavioral characteristics observed (Table 1). With increasing dose, the animals generally exhibited significantly more serotonin-related behavior (Table 2A) The frequency of all behavior measurements except two paws on the wall and grooming increased in a dose-dependent manner as the fenfluramine dosage increased Head weaving was the most sensitive behavioral measurement relative to the level of fenfluramine administered

TABLE 2D

EFFECT OF FENFLURAMINE, DIETARY TRYPTOPHAN AND PYRIDOXINE INTERACTION ON BEHAVIORAL MEASUREMENTS

Fenfluramine (mg/kg)	Tryptophan (% of diet)	Pyridoxine HCl (mg/kg diet)	Backward Walking	Front Paw Treading	Head Weaving	Hind Limb Abduction	Straub Tail	Circling	Two Paws on Wall	Grooming
5	0 15	7	1 6	4 9	2 2	0 5	0 4	4 5	3 5	1 8
5	0 15	3,000	6 8	10 0	17 2	0 9	3 3	6 4	14 5	1 6
5	0 6	7	5 3	10 2	6 1	1 5	1 2	8 1	14 5	6 2
5	0 6	3,000	1 8	13 5	50 3	1 5	1 5	12 8	4 9	2 9
10	0 15	7	5 3	17 2	27 2	0 5	2 5	20 9	3 4	3 1
10	0 15	3,000	19 0	22 4	63 6	3 0	8 3	16 8	7 3	4 5
10	0 6	7	7 9	24 7	36 2	1 2	1 2	21 4	10 5	4 8
10	0 6	3,000	9 5	28 5	42 7	1 2	4 9	23 5	11 6	2 8
15	0 15	7	3 9	27 8	74 9	0 5	5 9	12 8	6 2	2 5
15	0 15	3,000	13 1	31 4	62 1	3 0	5 6	5 6	6 6	0 4
15	0 6	7	15 6	16 8	68 2	0 7	12 5	38 8	13 1	4 5
15	0 6	3,000	19 4	13 8	51 5	1 8	13 8	26 5	5 5	2 8
20	0 15	7	16 0	24 7	122 0	0 7	5 9	18 1	3 1	2 2
20	0 15	3,000	18 5	20 9	94 5	2 2	7 1	30 6	6 1	2 4
20	0 6	7	12 5	33 7	86 1	8 1	13 5	21 4	5 8	5 0
20	0 6	3,000	18 1	21 9	73 1	4 5	15 2	20 4	6 1	1 3

In general, although it did not reach statistical significance in all but one instance, there was a higher frequency of the majority of the observed behaviors in the rats that had been fed the high tryptophan diets (Table 2B) This phenomenon was most visible at the lower fenfluramine (5 mg and 10 mg/kg) dosages administered Frequency of circling behavior was significantly increased on the higher as compared to the lower dietary tryptophan intake A dietary pyridoxine effect was most discernible with head weaving and hind limb abduction (Table 2C)

An interaction among fenfluramine, tryptophan and vitamin B_6 in relation to several behavior characteristics was noted when low doses of fenflurmaine were administered (Table 2D) With the 5 mg/kg dose of fenfluramine, frequencies of front paw treading, head weaving and hind limb abduction varied with the dietary treatment Frequencies of these behavior traits were lowest in the control and highest in the group fed the high tryptophan plus high vitamin B_6 diet At higher fenfluramine levels (15 and 20 mg/kg), differential dietary effects were no longer observed Head weaving was the most sensitive of the behavioral traits in relation to dietary treatment Interestingly, the effect of diet on this behavioral measurement was completely reversed at the highest fenfluramine dose administered (20 mg/kg)

The appearance of flushing was noted unexpectedly while the other behavioral observations were being made The presence or absence of flushing was dependent on the dosage of fenfluramine administered (Table 3A) At the 5 mg/kg dose of fenfluramine, no flushing appeared in any of the rats Dietary tryptophan level also significantly affected the frequency of flushing (Table 3B), with more animals experiencing flushing on the higher tryptophan intake. Dietary pyridoxine, on the other hand, did not influence the appearance of flushing (Table 3C)

Serotonin and 5HIAA concentrations were measured in the hypothalamus of the rats observed for behavioral

TABLE 3

CHI SQUARE ANALYSIS OF FLUSHING RESPONSE

	No	Yes
A Fenfluramine Dose (mg/kg)		
5	16 (100%)	0 (0%)
10	11 (69%)	5 (31%)
15	5 (31%)	11 (69%)
20	3 (19%)	13 (81%)
$\chi^2=26\ 42,\ p<0\ 000$		
B Tryptophan Level (% of diet)		
0 15	22 (69%)	10 (31%)
0 6	13 (41%)	19 (59%)
$\chi^2=5\ 11,\ p=0\ 024$		
C Pyridoxine HCl Level (mg/kg diet)		
7	20 (63%)	12 (37%)
3,000	15 (47%)	17 (53%)
$\chi^2=1\ 58,\ p=0\ 209$		

TABLE 4

POST-FENFLURAMINE 5HT AND 5HIAA CONCENTRATIONS IN HYPOTHALAMUS

Diet Group*	Saline Control	Fenfluramine (mg/kg body weight) 5	10	15	20
			A Serotonin Levels (μg/g tissue)		
I	0 35 ± 0 04*	0 35 ± 0 03*	0 28 ± 0 02*†	0 23 ± 0 03†	0 24 ± 0 02†
II	0 55 ± 0 03‡	0 30 ± 0 03*	0 26 ± 0 02*†	0 21 ± 0 01†	0 22 ± 0 02†
III	0 65 ± 0 02§	0 39 ± 0 04*	0 19 ± 0 02†	0 24 ± 0 02†	0 18 ± 0 04†
IV	0 54 ± 0 01‡	0 47 ± 0 03‡	0 23 ± 0 02†	0 18 ± 0 04†	0 28 ± 0 02*†
			B 5HIAA Levels (μg/g tissue)		
I	0 58 ± 0 01*	0 32 ± 0 01†	0 33 ± 0 03†	0 38 ± 0 02†	0 47 ± 0 01‡
II	0 65 ± 0 07*†	0 46 ± 0 03‡	0 37 ± 0 02†	0 36 ± 0 01†	0 45 ± 0 01‡
III	0 71 ± 0 03§	0 50 ± 0 02‡	0 24 ± 0 03†	0 41 ± 0 02†‡	0 42 ± 0 02†‡
IV	0 58 ± 0 01*	0 47 ± 0 02‡	0 36 ± 0 03†	0 43 ± 0 01†‡	0 41 ± 0 01†‡

Diet groups I (0 15% TRP, 7 mg Vitamin B_6), II (0 15% TRP, 3,000 mg Vitamin B_6), III (0 6% TRP, 7 mg Vitamin B_6), IV (0 6% TRP, 3,000 mg Vitamin B_6)

Mean ± S E (n=4)

Values with different superscripts are statistically significantly different from each other ($p<0$ 05, Duncan's Multiple Range Test)

changes following fenfluramine injection (Table 4) In comparison with control animals (saline injected), serotonin concentrations in all dietary treatment groups decreased inversely in animals injected with fenfluramine in a dose-dependent manner, with the lowest serotonin levels associated with the higher doses of fenfluramine In animals injected with 5 mg/kg fenfluramine, mean serotonin values were different, though not significantly so, in relation to the dietary tryptophan and/or vitamin B_6 level Rats on high levels of both tryptophan and vitamin B_6 had the highest serotonin values The dietary difference in serotonin levels was no longer visible when higher doses of fenfluramine were administered

The concentration of 5HIAA in the rat hypothalamus followed a pattern similar to that observed for the serotonin concentrations Tissue 5HIAA levels in fenfluramine injected rats decreased compared to control animals injected with saline only The decrease was not as pronounced as that for the serotonin levels The smallest decrease was seen in animals treated with the lowest (5 mg/kg) dosage of fenfluramine Interestingly, after reaching the lowest values at a 10 mg/kg dose, the 5HIAA concentrations increased again with 15 and 20 mg/kg fenfluramine administration

The fenfluramine dose had a greater impact in altering hypothalamic 5HIAA levels than serotonin levels When animals were injected with the 5 mg/kg dose, a small but non significant, differential dietary effect was visible At doses higher than 5 mg/kg, the dietary effect was completely absent

DISCUSSION

The results of the fenfluramine-induced, serotonin-mediated, behavioral observations indicate a clear tryptophan-vitamin B_6 interaction in relation to certain behavior traits in the rat Dietary tryptophan and vitamin B_6 levels, as well as fenfluramine dosage influenced the expression of serotonin-mediated behavioral traits and the appearance of flushing

The differential dietary effects on behavior were most visible upon usage of a low dose (5 mg/kg) of fenfluramine The frequencies of serotonin-mediated behavioral traits were lowest in the control animals and highest in the rats fed the high tryptophan plus high vitamin B_6 diet Separate effects of dietary tryptophan and vitamin B_6 were also detectable, but only when low doses of fenfluramine were administered However, at higher fenfluramine levels (15 and 20 mg/kg), such dietary effects were no longer discernible

Among the behavioral traits observed, head weaving was the one most sensitive to both dietary treatment (tryptophan or vitamin B_6) or fenfluramine dosage, confirming reports of other workers [5,31] In this study, high levels of dietary tryptophan or of vitamin B_6 as well as the administration of high doses of fenfluramine caused significant increases in the frequency of head weaving

Head weaving has been reported to be very sensitive to serotonergic manipulation Corne *et al* [5] quantified the occurrence of the head movement response following an intraperitoneal injection of 5-hydroxytryptophan (5HTP) (a serotonin precursor) in mice and used this as an index of the functional activity in serotonergic synapses Several other workers have used the head movement response to study serotonergic transmissions [18] Drugs, as well as serotonin precursors that increase the serotonergic transmission have been shown to increase the frequency of head weaving significantly [18]

It is not known why increased serotonergic transmission produces increased head movement but it has been reported that the serotonin (5HT) $5HT_2$-receptor subtype is involved in eliciting such a response [29] Lucki *et al* [20] hypothesized that in the rat, the head twitch response in contrast to other serotonin-related symptoms, is mediated by $5HT_2$ instead of $5HT_1$ receptors, respectively Moser and Redfern [22] studied the circadian variation in behavioral responses

to central 5HT-receptor stimulation They found that only head twitch responses, but not other serotonin-mediated behavior showed a circadian variation, offering experimental evidence supporting the existence of different 5HT-receptor subtypes

The combined treatment of high doses of fenfluramine with high tryptophan intake reduced the frequency of the head weaving response in this study Martin *et al* [21], recently examined the head twitching response in relation to 5HTP injected intraperitoneally They reported that, depending on the dose used, either an increase or decrease in head twitching response occurred At higher 5HTP it was decreased

Two of the other behavioral traits observed in this study, backward walking and circling, have been considered to be mediated by a simultaneous release of dopamine and serotonin Curzon *et al* [6] found that either amphetamine or fenfluramine at higher dosage (15–30 mg/kg), provoked backward walking and tight circling, which is a dopamine-mediated behavioral pattern Similar observations for these behaviors have also been described following high doses of p-chloroamphetamine, a releaser of endogenous serotonin [29] These results are interpreted to signify that high doses of fenfluramine can cause a release of catecholamines as well as of serotonin Increases in circling behavior exhibited by animals injected with high doses (15 and 20 mg/kg) of fenfluramine might have resulted from the simultaneous release of both serotonin and dopamine

The low dose of fenfluramine (5 mg/kg) permitted the observation of differential dietary effects on behavior However, at higher fenfluramine levels (15 and 20 mg/kg), such differential dietary effects were no longer detectable Conceivably, at the higher levels, the amount of serotonin released exceeded the minimum concentrations needed to differentially affect behavior patterns thus precluding the observations of diet-induced differences

The appearance of flushing was dependent on both dietary tryptophan intake and the dosage of administered fenfluramine, but not on vitamin B_6 intake Serotonin plays a role in the regulation of blood flow and central serotonergic pathways are involved in the regulation of arterial blood pressure [11,33] In the peripheral vessels, serotonin can cause contraction of blood vessels by its direct action on smooth muscle or by potentiating the effect of other vasoconstrictor agents It has been demonstrated that serotonergic neurons in the central nervous system can both inhibit and facilitate central sympathetic activity. Some researchers have shown that serotonin injected intraventricularly can cause an increase in blood pressure, and others have found that it can decrease blood pressure [13]

The concentrations of hypothalamic serotonin and 5HIAA found at the completion of the study were dependent on the dosage of fenfluramine administered as well as on the earlier dietary treatment With a fenfluramine dosage of 5 mg/kg, mean serotonin values for the experimental groups were significantly different from each other, lowest in the control and highest in the high tryptophan, high B_6 group However, such differential dietary effects disappeared when higher doses of fenfluramine were administered Concentrations of 5HIAA were lower with 5 and 10 mg/kg fenfluramine, and higher at fenfluramine levels of 15 and 20 mg/kg This finding suggests an increased turnover of serotonin at the higher fenfluramine levels

Interestingly, even the highest dose of fenfluramine administered did not completely deplete hypothalamic serotonin storage There have been other studies that have reported similar values for serotonin in animals injected with fenfluramine or with a serotonin neurotoxin such as 5,7-dihydroxytryptamine [5,10] In these studies, fenfluramine or 5,7-dihydroxytryptamine injection decreased brain serotonin levels by more than 50%, but did not deplete it completely Some serotonin, possibly bound, appears to be present at all times, not susceptible to the action of a releasing compound such as fenfluramine There might also be two types of storage sites for endogenous serotonin, the one more liable to manipulation through drugs such as fenfluramine, and the other, resistant to such treatment The location and functional significance of the remaining serotonin is worthy of further investigation

The changes in serotonin concentration following fenfluramine treatment corresponded well with the behavioral changes noted With a low dose of fenfluramine (5 mg/kg) the amount of serotonin released into the synapse appeared to reflect the amount of serotonin stored in the granules, which, in turn, depended upon the dietary level of tryptophan and vitamin B_6 High doses of fenfluramine induced a maximum release of available (releasable) serotonin, such that both the biochemical and behavioral values indicated the complete abolishment of differential dietary effects

It would have been of interest to have tested a level of fenfluramine lower than 5 mg/kg This might have permitted the expression of changes in the serotonin level with the dosage of fenfluramine in a dose-dependent manner It is also possible that a yet more definitive dietary effect on brain serotonin and 5HIAA levels might have been observed under such treatment conditions

CONCLUSIONS

The present study has demonstrated a clear interaction between dietary tryptophan and pyridoxine on certain serotonin-mediated behavioral traits in rats Upon administration of a low dose of fenfluramine, differential dietary effects were demonstrable The behavior head weaving was the most sensitive trait in response to the dietary tryptophan plus pyridoxine and fenfluramine interaction The post-fenfluramine neurochemical data corresponded well with the behavioral observations The appearance of flushing, a newly observed characteristic, was also dependent on dietary tryptophan intake and the dosage of administered fenfluramine, but not on vitamin B_6 intake

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