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Dietary Pyridoxine Interaction With Tryptophan or Histidine on Brain Serotonin and Histamine Metabolism[1]

NAM SOO LEE,* GEORGIA MUHS,* GEORGE C. WAGNER,†
ROBERT D. REYNOLDS‡ AND HANS FISHER*[2]

**Departments of Nutrition and †Psychology*
Rutgers, The State University of New Jersey, New Brunswick, NJ 08903
and ‡Beltsville Human Nutrition Research Center, USDA, Beltsville, MD 20705

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LEE, N S , G MUHS, G C WAGNER, R D REYNOLDS AND H FISHER *Dietary pyridoxine interaction with tryptophan or histidine on brain serotonin and histamine metabolism* PHARMACOL BIOCHEM BEHAV **29**(3) 559–564, 1988 —We studied the metabolic effects of high dietary intakes of pyridoxine and of the substrate-cofactor interaction between dietary histidine or tryptophan and pyridoxine in rat brain In the substrate-cofactor interaction study, histamine and serotonin levels were determined in rats fed elevated or requirement levels of substrate (histidine 0 3% and 0 8%, tryptophan 0 15% and 0 6%) and excess or requirement levels of pyridoxine HCl (7 mg vs 3,000 mg/kg) Excess pyridoxine intake caused a differential effect on brain histamine concentration—inhibitory with the requirement level of histidine (−29%), and stimulatory (+21%) with the elevated level of histidine When dietary tryptophan was fed at the requirement level, excess pyridoxine caused essentially no changes in hypothalamic serotonin and 5HIAA (−2%, −2%) With elevated tryptophan intake, excess pyridoxine significantly increased serotonin and 5HIAA (+32%, +20%) in the hypothalamus These results indicate a clear interaction between substrate and coenzyme precursor which influences brain metabolism of histamine and serotonin

Histamine Serotonin Pyridoxine Brain Tryptophan Histidine Vitamin B_6 Hypothalamus

VERY high intakes of vitamin B_6 have been shown to produce neurotoxic effects on sensory neurons in animals [17, 29, 30] A similar neurotoxic effect of high intakes of pyridoxine has been reported recently in several clinical cases [1, 26, 31]. In the human study, the authors did not find central nervous system toxicities which would account for their observations As to the mechanisms of the neurotoxic effect of pyridoxine, it was postulated that high levels of pyridoxine competitively inhibit pyridoxal-5-phosphate (PLP) in its binding to PLP-dependent apoenzymes

Vitamin B_6, in the form of the coenzyme PLP, plays an important role in the metabolism of numerous biologically important compounds Two compounds of special interest, histamine and serotonin, are synthesized by the decarboxylase enzymes, histidine decarboxylase (HDC) and 5-hydroxytryptophan decarboxylase (5HTPDC), both of which require PLP as a cofactor.

The influence of vitamin B_6 on the central nervous system has been extensively studied [7,8] A definitive role for vitamin B_6 in the central nervous system has been demonstrated through vitamin B_6-deficiency studies which produced biochemical and neurological changes [4, 12, 20]

PLP levels in tissues other than brain have been shown [14] to change in response to varying the vitamin B_6 intake over a relatively small range In brain, however, much smaller changes have been shown to occur from such dietary treatment No one to date appears to have examined the influence of high intakes of dietary vitamin B_6 on brain levels of PLP

In addition to influencing brain PLP availability, diet also can influence neuronal metabolism of serotonin or histamine through alterations in the availability of the transmitter precursors, histidine or tryptophan [2]. We have previously shown that an elevated dietary intake of histidine increased the activity of HDC and the concentration of histamine in various rat tissues including the brain [18].

It is also well established that elevated dietary tryptophan levels can increase brain levels of tryptophan followed by changes in concentrations of serotonin and its metabolite, 5-hydroxyindoleacetic acid (5HIAA), thus affecting serotonergic transmission [10,11] These changes have been shown in animals fed levels of dietary tryptophan (1–2% of diet) 5 to 10 times the requirement for this amino acid. We are not aware of studies in which the tryptophan intake was

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[2]Requests for reprints should be addressed to Hans Fisher

varied over a more narrow physiological range and which examined the interaction, synergistic or antagonistic, between the substrate (amino acid) and the cofactor precursor (pyridoxine)

The objectives of the present study were to explore (1) the effect of dietary vitamin B_6 supplementation on brain metabolism of histamine, serotonin and pyridoxine, (2) the substrate and coenzyme interaction between histidine or tryptophan and vitamin B_6 and its effect on the metabolism of histamine and serotonin The ultimate goal of the present investigation was to study the possibility that some of the reported neurotoxic effects of a very high vitamin B_6 intake might result in changes in brain neurotransmitter levels that are reflected in behavioral consequences

METHOD

Animals

Sprague Dawley, C/D male weanling rats (body weight 50–60 g from the 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 with free access to the experimental diets and to water The animals were regularly weighed and their food consumption recorded After 10 days of being fed the experimental diets, they were sacrificed by decapitation between 8 00 and 10 00 hr (one to three hours after the lights had turned on). Brain tissues were immediately removed, regions of the brain separated, frozen in liquid nitrogen and stored at −80°C until the time of assay, always less than two weeks

Diets

For the substrate-cofactor interaction studies between histidine or tryptophan and vitamin B_6, semi-purified diets were given to the animals The semi-purified experimental diets provided the minimum daily requirement of protein plus amino acids (13%) for young growing rats All experimental diets were isocaloric and isonitrogenous with one another The experimental diets provided two levels of histidine (0 3, 0 8%) or tryptophan (0 15, 0 6%), and two different levels of pyridoxine HCl (7, 3,000 mg/kg diet) The lower levels of amino acids represented the reported [25] National Research Council (NRC) requirement level for young growing rats and the higher levels were chosen to be in the range of concentrations found in commercial rat diets The low level of pyridoxine HCl (7 mg/kg) represented the reported [25] NRC recommended level (6 mg/kg) The high level (3,000 mg/kg) of pyridoxine was approximately 400 times that of the lower level

DIETARY VARIABLES FOR INTERACTION STUDIES

Pyridoxine HCl (mg/kg diet)	Histidine (% of diet)	Tryptophan (% of diet)
7	0 3	0 15
3,000	0 8	0 6

The composition of the experimental diets is listed in Table 1

Reagents

Aminoguanidine sulfate, pyridoxal-5-phosphate, histamine dihydrochloride, serotonin creatinine sulfate and 5-hydroxyindoleacetic acid were purchased from Sigma Chemical Company (St Louis, MO) L-1-^{14}C-histidine (specific activity, 55 9 mCi/mmol), ^{14}C-S-adenosylmethionine (specific activity, 58 3 mCi/mmol) and L-1-^{14}C-tyrosine (32 4 μCi/mM) were obtained from New England Nuclear (Boston, MA)

TABLE 1

COMPOSITION OF EXPERIMENTAL DIETS

Ingredients	Amount %
Casein, edible grade	11 8
L-Arginine	0 2
L-Methionine	0 25
L-Threonine	0 07
L-Tryptophan	0 or 0 45
L-Histidine	0 or 0 51
Glycine*	0 45–0 96
Corn Starch	57 8
Sucrose	5 0
Glucose	10 0
Mineral Mix, MM†	4 0
Cellulose	4 0
AIN-76 Vitamin Mix‡	1 0
Additional Pyridoxine HCl§	0 or 3,000 mg/kg
Choline Chloride	0 2
Corn Oil	5 0

*The low histidine diet supplied 0 96%, the low tryptophan diet supplied 0 90%, the high histidine and high tryptophan diets supplied 0 45%

†Composition of mineral mix (mg element/kg diet) Ca (4,491), P (2,997), K (3,746), Mg (375), Fe (38), I (0 31), Mn (81 1), Zn (25 9), Cu (15 3), Na (4,342 4), Cl (6,677 5), Se (0 27), Mo (1 12), Cr (0 49), B (0 35), V (0 22), Sn (1 05), As (1 2), Si (15 7), Ni (3 0), F (2 71), Co (0 2)

‡Composition of AIN-76 vitamin mix (g/kg) Thiamine HCl (0 6), Riboflavin (0 6), Pyridoxine HCl (0 7), Niacin (3 0), Calcium pantothenate (1 6), Folate (0 2), Biotin (0 02), Vitamin B_{12} (1 0), Vitamin A palmitate (500,000 U/g, 0 8), Vitamin D_3 (400,000 U/g, 0 25), Vitamin E acetate (500 U/g, 0 8), Menadione sodium bisulfite (0 08), Sucrose, finely powered (981 15)

§For the high B_6 diets, 3,000 mg pyridoxine HCl were mixed with 1 kg of diet

Biochemical Analyses

Tissue histamine concentrations were determined radioenzymatically by the procedure of Taylor and Snyder [34] with the modifications described by Ishibashi *et al* [13] Tissue protein concentrations were assayed by the Lowry method [21]

Serotonin and 5HIAA concentrations were analyzed by an HPLC-electrochemical detection method [24]. The HPLC unit used was a Bioanalytical System LCEC Analyzer (BAS, Model LC-150) A C_{18} reverse-phase column (Biophase, 5μ particle size, 250×4 6 mm i d) and an LC-4B electrochemical detector were attached to the HPLC unit The mobile phase was sodium phosphate, citrate buffer containing 15% HPLC-grade methanol, and the flow rate was 1 0 ml/minute The oxidative potential was set at +0 72 V and the detection range was set at 10 nA.

Hypothalamic PLP levels were determined by the tyrosine apodecarboxylase assay developed by Lumeng *et*

TABLE 2

DIETARY HISTIDINE × VITAMIN B_6 INTERACTION—WHOLE BRAIN HISTAMINE CONCENTRATIONS

Measurement	Low HIS Normal Vitamin B_6	Low HIS High Vitamin B_6	High HIS Normal Vitamin B_6	High HIS High Vitamin B_6
Whole Brain Histamine (nmol/g tissue)	1 27 ± 0 08[a]	0 90 ± 0 07[b]	1 57 ± 0 13[c]	1 90 ± 0 16[d]

Values Mean ± S E (n=12)
Low HIS 0 3% (NRC recommended level)
High HIS 0 8%
Normal Vitamin B_6 7 mg/kg (NRC recommended level)
High Vitamin B_6 3,000 mg/kg
Values with different superscripts are statistically significantly different at $p<0$ 05

TABLE 3

DIETARY TRYPTOPHAN × VITAMIN B_6 INTERACTION—HYPOTHALAMIC SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID CONCENTRATIONS

Hypothalamic Measurement	Low TRP Normal Vitamin B_6	Low TRP High Vitamin B_6	High TRP Normal Vitamin B_6	High TRP High Vitamin B_6
Serotonin (μg/g tissue)	0 30 ± 0 02[a]	0 33 ± 0 01[a]	0 48 ± 0 01[b]	0 63 ± 0 05[c]
5-Hydroxyindoleacetic acid (μg/g tissue)	0 21 ± 0 02[a]	0 20 ± 0 02[a]	0 49 ± 0 03[b]	0 58 ± 0 02[c]

Values Mean ± S E (n=18)
Low TRP 0 15% (NRC recommended level)
High TRP 0 6%
Normal Vitamin B_6 7 mg/kg
High Vitamin B_6 3,000 mg/kg
Values with different superscripts are statistically significantly different at $p<0$ 05

al [22] as modified by Reynolds [27]. Homogenization was carried out by the needle and syringe technique (Multifit glass syringe, 5 ml size, 22 gauge, 1'' needle) by withdrawing and expelling forcefully 6–7 times until uniformly homogenized

Statistical Analysis

The results of the experiments on the dietary histidine or tryptophan interaction with pyridoxine were analyzed by analysis of variance, using a SAS program [28]

RESULTS

Body Weight Gain and Food Intake

Body weight gain and food intake did not vary significantly among the experimental treatments for all experiments suggesting that the excess vitamin B_6 was not detrimental to the growth of the rats

Interaction Between Dietary Histidine and Vitamin B_6

Dietary histidine level influenced brain histamine concentration in confirmation of previous work from this laboratory [18] There was a 34% increase in whole brain histamine concentration in rats receiving 0 8 compared to 0 3% dietary histidine (Table 2) The high vitamin B_6 intake decreased the brain histamine level by 29% in rats getting 0 3% histidine In combination with the higher histidine intake (0 8%) the high dietary vitamin B_6 increased brain histamine concentration by 149% when compared to the level in rats fed the control level of vitamin B_6 (7 mg/kg) and histidine (0 3%), and by an additional 21% when compared to the rats fed 0.8% histidine and the control level of vitamin B_6

Interaction Between Dietary Tryptophan and Vitamin B_6

Hypothalamic concentrations of serotonin and 5HIAA were increased by increased dietary tryptophan intake (Table 4), confirming the reports of other workers [10,11]. In this study, the dietary precursor effect on brain serotonin concentration was demonstrated by comparing two diets that provided tryptophan at levels not exceeding those found in "normal" commercial or purified diets The higher tryptophan diet (0 6%) significantly increased the serotonin and 5HIAA concentrations by 61 and 131%, respectively (Table 3) in comparison with the concentrations observed on the 0 15% tryptophan diet Unlike the findings with histidine, excess pyridoxine intake caused essentially no change in serotonin and 5HIAA concentrations at the 0 15% dietary tryptophan level The diet providing the higher level of tryptophan and excess pyridoxine significantly increased the concentrations of serotonin and of 5HIAA by 113 and 175%, respectively, in comparison with the lower tryptophan diet The pyridoxine effect, superimposed upon the higher tryptophan (0 6%) intake alone, resulted in a 32 and a 19% increase, respectively, for serotonin and 5HIAA

TABLE 4
HYPOTHALAMIC PLP CONCENTRATION

Measurement	Low HIS Normal Vitamin B_6	Low HIS High Vitamin B_6	High HIS Normal Vitamin B_6	High HIS High Vitamin B_6
A Dietary Histidine × Vitamin B_6 Interaction				
Hypothalamic PLP (nmol/g tissue)	4 22 ± 0 13	3 96 ± 0 04	3 76 ± 0 15	3 95 ± 0 17
B Dietary Tryptophan × Vitamin B_6 Interaction				
Measurement	Low TRP Normal Vitamin B_6	Low TRP High Vitamin B_6	High TRP Normal Vitamin B_6	High TRP High Vitamin B_6
Hypothalamic PLP (nmol/g tissue)	3 90 ± 0 11	3 90 ± 0 06	3 86 ± 0 11	3 74 ± 0 09

Values Mean ± S E (n=6)
Mean PLP values for both groups A and B were not significantly different from each other
Low HIS 0 3% (NRC recommended level)
High HIS 0 8%
Low TRP 0 15% (NRC recommended level)
High TRP 0 6%
Normal Vitamin B_6 7 mg/kg (NRC recommended level)
High Vitamin B_6 3,000 mg/kg

Concentrations of PLP in the Hypothalamus

PLP levels in the hypothalamic tissues of rats from the histidine × vitamin B_6 and tryptophan × vitamin B_6 interaction studies are listed in sections A and B of Table 4 PLP concentrations on the high pyridoxine diets did not vary significantly when compared to normal-pyridoxine-containing diets Neither dietary histidine nor tryptophan level influenced the concentration of PLP in the hypothalamus When the PLP values from all animals fed the same pyridoxine diet (either normal or high) were pooled together, there was still no difference in PLP concentrations between the two diets providing sharply different levels of pyridoxine HCl (7 vs 3,000 mg)

DISCUSSION

There was a differential effect of high vitamin B_6 feeding on histamine metabolism in the brain Excess vitamin B_6 when added to diets supplying 0 3% histidine caused a significant depression in brain histamine concentration (Table 2) Essentially no change in hypothalamic serotonin concentration occurred when excess pyridoxine was added to a diet providing 0 15% (the requirement) tryptophan (Table 3) Excess vitamin B_6, in combination with higher, but not unusually so, intakes of histidine (0 8%) and tryptophan (0.6%), significantly increased brain histamine and serotonin concentrations (Tables 2 and 3) These findings indicate that not only precursor substrate (histidine or tryptophan) or coenzyme precursor (pyridoxine) independently, but their combination as well, can influence brain concentrations of histamine and serotonin.

It has been previously shown that additions *in vitro* or *in vivo* of high concentrations of PLP inhibit brain HDC or 5HTPDC activities [23] According to these studies, PLP forms complexes with histamine or histidine, and with tryptophan or serotonin These complexes may be formed by the direct action of the amino acids or amines with PLP, or they may be formed from a Schiff base which undergoes cyclization Increases in the formation of such a cyclization product between histidine and PLP or between histamine and PLP have been previously quantitated [15,36] A similar inhibitory effect of high PLP in the brain has also been reported recently in another PLP-requiring enzyme system, glutamic acid decarboxylase and its product, γ-aminobutyric acid (GABA), in seizure-prone mice injected with PLP High levels of PLP injected intraperitoneally or intracerebroventricularly caused epileptic seizures which were preventable by GABA or by feeding pyridoxine-deficient diets to these animals [9, 16, 35]

Despite these observations, the mechanism as to how excess pyridoxine intake decreases histamine levels is difficult to explain

In the presence of high levels of dietary histidine or tryptophan, excess vitamin B_6 increased the concentration of histamine and serotonin synergistically, which is also difficult to explain by the mechanism of cyclization If cyclization is the cause of depressed histamine formation at lower levels of histidine intake, there must be a fine line between this process and the stimulation of HDC to form more histamine in the presence of higher levels of histidine

In an effort to explain the differential effect of excess pyridoxine in these studies, brain PLP concentrations were analyzed in the histidine × vitamin B_6 and tryptophan × vitamin B_6 interaction studies Surprisingly, the concentrations of PLP in hypothalamic tissues of rats on the high pyridoxine diets did not vary significantly when compared to those fed a normal pyridoxine-containing diet Neither did dietary histidine nor tryptophan level influence hypothalamic PLP concentrations The synergistic effect between the high levels of histidine or tryptophan and excess vitamin B_6 thus cannot be explained by changes in brain PLP concentration

PLP concentrations in tissues other than brain have been shown to undergo considerable change upon varying the vitamin B_6 intake. By contrast, much smaller changes have been shown to occur in brain from varying the pyridoxine intake. Spector [32,33] noted that high dietary levels of pyridoxine inhibited the uptake of pyridoxine by the brain, and accelerated dephosphorylation, keeping the concentration of brain PLP relatively constant. Brain PLP level, however, has been shown to change in certain circumstances such as in severe vitamin B_6 deficiency or in pathological conditions of the central nervous system. Dakshinamurti *et al* [5,6] demonstrated lowered levels of PLP and brain biogenic amines in animals on pyridoxine-deficient diets. A more than 50% increase in PLP has been demonstrated in brains 30 minutes after an intraperitoneal injection of PLP into seizure-prone rats [9,16]. Chung *et al* [3] reported a transient increase in whole brain PLP following a withdrawal period in alcohol-intoxicated mice, which lasted 48 hours.

In the present study, the levels of hypothalamic PLP did not vary with excess pyridoxine intake. It is possible that the observed biochemical changes in histamine or serotonin concentration might have resulted from changes in other metabolites of pyridoxine. We did not measure other B_6 vitamers in the brain or in other tissues. Perhaps these other pyridoxine metabolites play a role.

We also do not know whether the responses to dietary pyridoxine on brain histamine and serotonin concentration are dependent upon the specific level used in this study. Such effects might well occur with much lower doses of pyridoxine.

SUMMARY

The following summarizes the results of the present study:

(1) High dietary intake of pyridoxine caused a significant depression in histamine concentration in brains of rats fed diets supplying 0.3% histidine.

(2) High pyridoxine intake exerted a stimulatory effect on brain histamine when dietary histidine was raised from 0.3 to 0.8%.

(3) High pyridoxine intake did not affect hypothalamic serotonin and 5HIAA concentrations when the diet provided the NRC requirement level of tryptophan (0.15%). However, with tryptophan intake of 0.6% a high intake of pyridoxine increased serotonin and 5HIAA concentrations in the hypothalamus.

(4) Concentrations of PLP in the hypothalamus were not significantly changed when the diet supplied high pyridoxine. Thus, the observed biochemical changes in brain histamine and serotonin concentrations cannot be explained on the basis of changes in brain PLP concentrations.

(5) Behavioral consequences of such substrate-coenzyme precursor interactions as studied may be anticipated due to the neuronal roles of both histamine and serotonin in mammalian brain. Such observations form the subject of a subsequent study from this laboratory [19].

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