Effect of Vitamin B-6 Deficiency on Glutamic Acid Decarboxylase Activity in Rat Olfactory Bulb and Brain

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ABSTRACT The effect of dietary vitamin B-6 deficiency on L-glutamic acid decarboxylase (GAD) activity in the olfactory bulb and whole brain of young growing rats was measured. Male Charles River CD albino rats of similar age (23 days) and weight (40 to 50 g) were divided into three dietary groups: one was fed a purified control diet (2 mg pyridoxine·HCl/100 g diet) ad libitum (C); the second group (D) was fed an identical diet deficient in vitamin B-6 (15.3 μg pyridoxine·HCl/100 g diet); the third group (R) was fed the control diet in restricted amounts, paired to the average amount consumed by D. After 102 days of feeding the respective diets, some of the D rats were repleted by injecting (ip) 10 mg pyridoxine·HCl and subsequently feeding the control diet ad libitum for varying lengths of time. By measuring GAD activity with and without added pyridoxal phosphate (PLP) in the assay medium, it was shown that the reduced GAD activity in the olfactory bulb and whole brain tissues of D (approximately 50% of the control values) was due to cofactor depletion and not to a decreased synthesis of GAD protein. Although body weight was decreased by feeding restricted amounts of the control diet, none of the other parameters (olfactory bulb and whole brain GAD activity, serum glutamic oxalacetic transaminase (GOT) activity, urinary xanthurenic acid excretion) for R were significantly different from C. GAD activity measured without added PLP in the olfactory bulb and whole brain of D, as well as serum GOT activity and urinary xanthurenic acid excretion were affected after 5 days of feeding. The mean body weight of D was significantly lower than R after 2 weeks of feeding. Repletion quickly returned GAD activity in olfactory bulb and whole brain, serum GOT activity, and urinary xanthurenic acid excretion to control values. Body weights of the repleted rats increased dramatically and reached the mean weight of R. It was concluded that the GAD activity of the olfactory bulb and of the whole brain respond similarly to dietary vitamin B-6 deficiency. J. Nutr. *109:* 1694–1702, 1979.

INDEXING KEY WORDS B-6 deficiency · GAD activity · olfactory bulb · GOT activity · xanthurenic acid

Glutamic acid decarboxylase (L-glutamate 1-carboxy-lyase; EC 4.1.1.15, GAD) is the rate limiting enzyme in the synthesis of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. Subsequently, GAD activity determines steady-state levels of GABA in the central nervous system (1). GAD requires pyridoxal phosphate (PLP) as a co-

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factor for converting L-glutamic acid to GABA and carbon dioxide (1). It has long been recognized that GABA levels in rat whole brain are decreased during vitamin B-6 deficiency due to a depletion of cofactor which results in decreased GAD activity (2). More recently it has been shown, using immunohistochemical techniques, that GAD is localized in inhibitory neurons of rat olfactory bulb (3). The physiology of the neuronal network of the olfactory bulb demonstrates that these GABAergic neurons function as a filter for incoming sensory information. Such horizontal communication within the olfactory bulb is undoubtedly essential for following an intensity gradient of olfactory cues. Olfactory sensory information is important for mediating such behaviors as suckling in neonatal humans (4) and rats (5, 6), locating food sources in macrosmotic animals (7), and social interactions in a multitude of adult animal species (8).

Although most pyridoxine deficiency studies have addressed themselves to the delineation of the effects on whole brain GAD and to the role of GABA in convulsions and seizures (9), the effects of pyridoxine deficiency on olfactory bulb GAD have not been reported. The purpose of this study was to compare the effects of vitamin B-6 nutriture on the activity of olfactory bulb GAD with that of whole brain GAD in the young growing rat. The effects of vitamin B-6 deprivation on serum glutamic oxalacetic transaminase (GOT, aspartate aminotransferase, AST: EC 2.6.1.1) activity and urinary xanthurenic acid excretion were also measured. In addition, the rate at which the above parameters returned to normal was examined during a period of vitamin B-6 repletion.

MATERIALS AND METHODS

Animals and diets. Male, weanling, CD albino rats [3] (40 to 50 g) were individually housed in stainless steel cages equipped with a stainless steel food cup and two glass drinking bottles. Room temperature and relative humidity were held constant and a 12-hour light-dark cycle was maintained. All rats were fed a control purified diet for 2 days (adjustment period), weighed and divided into three dietary groups such that each group had approximately the same average body weight. The control dietary group (C) was fed ad libitum a complete purified diet consisting of the following (in %): vitamin free casein,[4] 25.0; cornstarch, 58.0; corn oil, 6.5; cellulose fiber, 3.0; mineral mix,[5] 5.0; and vitamin mix,[6] 2.5. The vitamin B-6 deprived group (D) was fed a diet identical to that of the control group except that the vitamin mix was devoid of pyridoxine·HCl. As a control for the diminished food intake of D, a third group (R) was fed the control diet in restricted amounts determined by the average amount of diet consumed by D. Body weight and food intake were measured every 2 to 3 days.

The rats used in these experiments were subsets of larger identical dietary groups which were used for an unrelated pyridoxine deficiency study. Body weight and urinary xanthurenic acid excretion data were derived in part from the larger groups of rats for statistical purposes. The experimental protocol was completed in two chronological studies to give a longitudinal insight into the development of the vitamin B-6 deficiency. The first set of experiments provided samples at time zero and after 17, 102 and 123 days of feeding the experimental diets. The effect of vitamin B-6 repletion was investigated after feeding the respective diets for 102 days. Rats from D were either maintained on the deficient diet during the 21 day repletion period, for a total of 123 days of feeding the deficient diet, or injected (ip) with 10 mg pyridoxine·HCl (20 mg/ml saline) and fed the control diet ad libitum for 2 minutes, 20 minutes, 24 hours or 21 days. Rats representing R and C were also injected (ip)

[3] Charles River Laboratories, Wilmington, Massachusetts.

[4] Obtained from ICN Pharmaceuticals, Inc., and contains 0.63 μg pyridoxine·HCl/100 g diet.

[5] Mineral mix (g/kg): ammonium molybdate·4 H_2O, 0.03; calcium carbonate, 292.9; calcium phosphate·2H_2O, 4.3; cupric sulfate, 1.56; magnesium sulfate·7H_2O, 99.8; manganese sulfate·H_2O, 1.2; potassium iodide, 0.005; potassium phosphate, 343.1; sodium chloride, 250.6; sodium selenite·5H_2O, 0.02; zinc chloride, 0.45; ferrous sulfate·7H_2O, 0.398.

[6] Vitamin mix (amount/100 g diet): pyridoxine·HCl, 2.0 mg; thiamin·HCl, 2.2 mg; riboflavin, 2.2 mg; nicotinic acid, 9.9 mg; calcium pantothenate, 6.6 mg; folic acid, 198 μg; choline chloride, 165 mg; vitamin (retinyl acetate) A, 1982 IU; ρ-aminobenzoic acid, 11 μg; inositol, 11 mg; vitamin B-12, 3 μg; ergocalciferol, 220 IU; D,L-α-tocopherol, 11 IU; biotin, 0.04 mg; menadione, 3 mg.

with the same dose of pyridoxine·HCl for comparison. The second set of experiments focused on the early phases of the deficiency (T_0, 5 and 8 days of feeding the respective diets). In the remaining text the data will be combined and considered continuous unless stated otherwise.

Xanthurenic acid and GOT assays. The rats were individually screened for 24-hour urinary excretion of xanthurenic acid (10) either with or without an injection (ip) of L-tryptophan (300 mg L-tryptophan/$kg^{0.75}$). Since the rats representing D were excreting such large amounts of xanthurenic acid in their urine, no tryptophan load was administered after the first 8 days of feeding. Rats representing each dietary group were killed at various times by decapitation. Blood was collected, centrifuged, and in most cases the serum was assayed for GOT activity using a colorimetric technique (11). The olfactory bulbs, and the whole brain minus the brainstem were immediately extirpated and placed in ice cold 50 mM potassium phosphate buffer (pH 6.8) containing 1 mM EDTA (dipotassium salt) and 1 mM AET (2-aminoethylthiouronium bromide hydrobromide). Homologous tissues were pooled for the respective dietary groups, blotted dry, weighed, and homogenized in 7 volumes of ice cold buffer using a Potter-Elvehjem tissue grinder with a Teflon pestle. The homogenates were centrifuged [7] at 35,000 × *g* for 15 minutes at 4° (12). The resultant supernatants were kept on ice and were immediately assayed for GAD activity and protein concentration.

GAD assay. GAD activity was assayed by a modification of the radiometric method described by Wilson et al. (12) which involves quantitating the $^{14}CO_2$ liberated from L-[1-^{14}C]glutamic acid during decarboxylation. The reaction medium (final volume 50 μl) consisted of 10 μl supernatant fluid diluted to approximately 2 mg protein/ml with the homogenizing (potassium phosphate) buffer, 4 μl 2.5 mM 2-mercatoethanol, 12 μl 50 mM potassium phosphate buffer (pH 6.8) containing 1 mM EDTA (dipotassium salt) and 0.5% (v/v) Triton X-100, 4 μl 1.25 mM pyridoxal phosphate (PLP) and 20 μl (1 μCi) L-[1-^{14}C]glutamic acid (S.A. 55 mCi/mmol) [8] in 2% ethanol. To obtain an estimate of in vivo haloenzyme activity the assays were also performed without the addition of PLP by substituting 4 μl deionized water for PLP in the assay medium. All assays were performed in quadruplicate.

GAD assays were carried out in 10 ml Erlenmeyer flasks equipped with a tight fitting rubber septum and a suspended plastic centerwell.[9] The centerwell contained a 2 × 2 cm fluted piece of Whatman No. 1 filter paper saturated with 0.1 ml 10% (w/v) degassed KOH (13). The reaction was initiated by addition of the enzyme preparation and each flask was incubated for 11 minutes in a water bath at 37° with mild shaking. The reaction was stopped by injecting 0.2 ml 10 mM acetic acid in methanol through the rubber septum into the reaction flask. Each flask was incubated for an additional 90 minutes and the center well was cut into a glass vial containing 10 ml diotol scintillation fluid (13). Blank assays were routinely performed during which the acetic acid in methanol and the enzyme were added simultaneously at time zero. Recovery of $^{14}CO_2$ was routinely measured by using Na_2 $^{14}CO_3$ (S.A. 50 mCi/mg) [10] in place of L-[1-^{14}C]glutamic acid and was always found to be 100 ± 5%. Vials were counted in a liquid scintillation spectrometer.[11] GAD activity was corrected for blanks and efficiency and is expressed as pmoles of product formed per mg protein per minute. Protein was measured by a modification of the Lowry method (14) using bovine serum albumin as standard.

Statistical analyses. Significant differences were determined by one-way analyses of variance and relied on the 95% confidence interval. Sample means were compared using Duncan's multiple range test (15) at the 5% level of significance ($P < 0.05$).

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[7] Sorvall RC2-B, Ivan Sorvall, Inc., Newton, Connecticut.
[8] Amersham/Searle Corporation, Arlington Heights, Illinois.
[9] Kontes Glass Co., Vineland, New Jersey.
[10] Amersham/Searle Corporation, Arlington Heights, Illinois
[11] Packard Tri-Carb Liquid Scintillation Spectrometer, Packard Instrument Company, Inc., Downers Grove, Illinois.

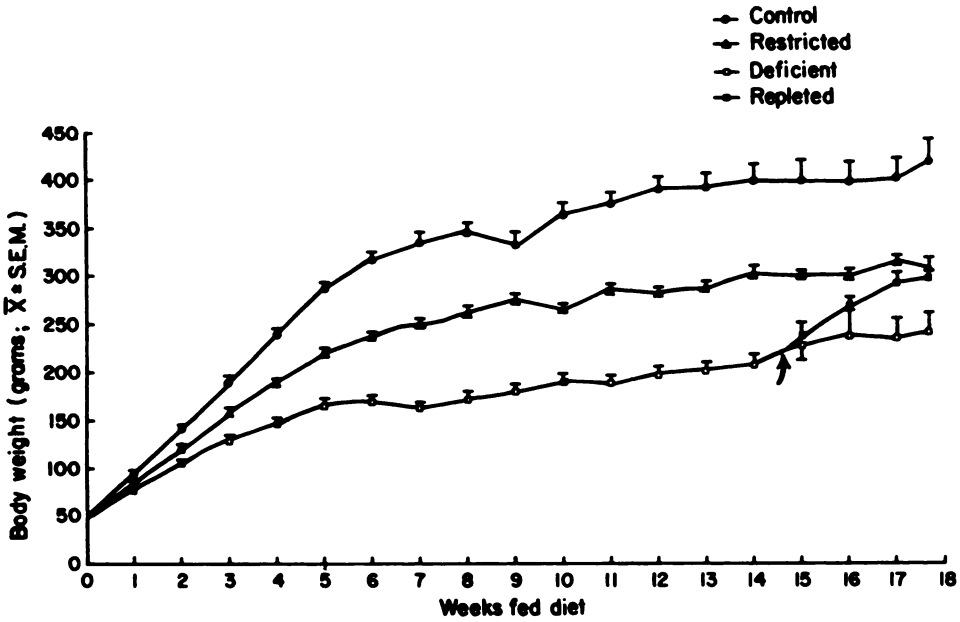

Fig. 1 The effect of vitamin B-6 deficiency on the body weight of rats. Each point represents the mean ± SEM of control rats ($n \geq 9$), food restricted rats ($n \geq 9$) and the deficient rats ($n \geq 9$). The arrow indicates the initiation of pyridoxine repletion in some of the deficient rats ($n = 8$).

RESULTS AND DISCUSSION

The rats fed the pyridoxine deficient diet exhibited the classical B-6 deficiency symptoms of a poor coat, sharp cries and acrodynia around the mouth, paws and tail. It is not surprising that convulsions and death were not observed since the B-6 deficient diet provided a trace of pyridoxine·HCl (see methods). The mean body weights of D, R and C were significantly different from each other after feeding the experimental diets for 2 weeks and remained so throughout the study (fig. 1). Repletion by intraperitoneal injection of 10 mg pyridoxine·HCl and feeding the control diet ad libitum for 10 days to deficient rats increased their mean body weight dramatically to a level significantly different from that of unrepleted D and C, but not different from R.

Serum GOT activity (table 1) of D was significantly decreased compared to that of C and R after 5 days of feeding the experimental diets and remained so throughout the study. Unlike the body weights, the GOT values of R were never different from that of C. Thus, the depressed body weights of R were undoubtedly due to calorie restriction and the amount of the pyridoxine consumed was adequate. Injection of 10 mg pyridoxine·HCl increased serum GOT activity within 20 minutes to a level similar to that of R. At 24 hours postinjection and after 21 days of repletion, the serum GOT activity of the repleted group was the same as that of the control. Table 1 also shows the amount of xanthurenic acid excreted during 24 hour periods in the filtered urine of individual rats representing each dietary group. The results are similar to the GOT data. After 5 days of feeding the experimental diets, D excreted significantly more xanthurenic acid than C or R and this persisted throughout the study. The amount of xanthurenic acid excreted by D after a tryptophan load seemed to increase between 5 and 8 days. Thereafter, the basal excretion level of xanthurenic acid of D remained relatively high. The amount of urinary xanthurenic acid excreted by R was never significantly different from that of C. Repletion for 21 days lowered the basal amount of xanthurenic acid excreted in the urine to a level

TABLE 1

The effect of vitamin B_6 deficiency on serum GOT activity and urinary excretion of xanthurenic acid

Days fed diet	Serum GOT activity		
	Deficient	Restricted	Control
	Sigma Frankel units/ml		
5	96 ±42[a,1] (2)	243 ± 8[b] (3)	203 ±21[b] (3)
8	111 ±10[a] (3)	189 ±10[b] (3)	195 ± 7[b] (3)
Repletion[2]			
2 min	144 ± 6[a] (2)		
20 min	173 ±20[a,b] (3)	243 ±10[b] (2)	
24 hr	253 ± 5[a] (3)	226 ±18[a] (2)	221 ± 3[a] (2)
21 days	216 ± 5[a] (8)	212 ±12[a] (9)	206 ± 8[a] (9)
123	102 ± 9[b]		
	Urinary xanthurenic acid excretion[3]		
	μg/ml		
5	36 ± 8.0[a,1] (7)	9.0± 3.0[b] (6)	6.6± 1.8[b] (8)
8	293 ±60.4[a] (8)	31.8± 7.8[b] (8)	37.6±16.4[b] (8)
17	255.8±37.6 (6)	3.8± 2.0[b] (8)	1.6± 1.4[b] (8)
123	187.6±56.0[a] (9)	8.6± 2.8[b] (9)	6.0± 2.4[b] (9)
Repletion[2]			
21 days	11.2± 4.6[b] (8)		

[1] Mean±SEM for duplicate analyses of number of samples in parentheses. Values within a row superscripted by the same letter are not significantly different at $P \leq 0.05$. [2] Rats representing each group were injected with 10 mg pyridoxine·HCl in saline; fed the control diet ad libitum, and sacrificed at the times indicated after injection. Comparisons are made among 2 and 20 minute samples, among the 24 hour samples, and among the 21 day repletion and 123 days of feeding the respective diets. [3] Rats representing each dietary group were injected with 300 mg L-tryptophan/Kg $B.W.^{0.75}$ before 24 hour urinary collections began after feeding the respective diets for 5 and 8 days. Thereafter, no tryptophan loads were administered prior to collection of 24 hour urinary samples.

similar to that of C and R and significantly less than that of unrepleted D.

The effects of dietary vitamin B-6 on whole brain GAD activity with and without PLP added to the assay medium are shown in table 2. The GAD values obtained for whole brain and olfactory bulb samples at the initiation (Day 0) of the two portions of this study (see methods) were essentially the same, thus they were pooled for each tissue. After feeding the experimental diets for 5 days, whole brain GAD activity without added PLP was significantly different among all three dietary groups. However, feeding restricted amounts of the control diet (R) did not appear to consistently affect whole brain GAD activity compared to C, whether PLP was added or not. The GAD activity of the whole brain samples from D, measured without the addition of PLP, was always significantly lower than that of R and C. It should be noted that addition of PLP to the GAD assay medium invariably increased GAD activity for whole brain (table 2) and for olfactory bulb samples (table 3). In some cases (8 and 102 days), the whole brain GAD activity of D in-

TABLE 2

The effect of vitamin B_6 deficiency on GAD activity in whole brain of rats

Days fed diet	Deficient		Restricted		Control	
	No added PLP	Added PLP	No added PLP	Added PLP	No added PLP	Added PLP
0					546 ±170[1] (7)	552 ± 73
5	298 ± 3[A,2] (3)	863 ±47[a]	491 ± 7[B] (3)	917 ±11[a]	529 ± 12[C] (3)	897 ± 2[a]
8	328 ±33[A] (3)	918 ±25[a]	496 ±11[B] (3)	780 ±10[b]	447 ± 19[B] (3)	839 ± 23[b]
17	245 ±15[A] (2)	877 ±234[a]	581 ±64[B] (2)	766 ±33[a]	680 ± 45[C] (2)	1,035 ± 8[a]
102	386 ±53[A] (2)	922 ±45[a]	432 ± 8[B] (2)	593 ± 7[b]	483 ± 13[B] (2)	644 ± 29[b]
Repletion[3]						
2 min	646 ±55[A,B] (2)	1,027 ± 22[a]	—	—	—	—
20 min	756 ±38[B]	1,334 ± 22[b]	548 ±19[A] (2)	727 ±23[c]	—	—
24 hr	682 ±15[A] (3)	841 ± 4[a]	487 ±31[A] (2)	689 ±12[b]	554 ± 76[A] (2)	713 ± 15[a]
21 days	683 ±44[A] (3)	947 ± 14[a]	662 ±71[A] (3)	991 ±11[b]	568 ± 35[A] (3)	951 ±109[b]
123	341 ±85[B] (3)	1,037 ± 48[b]				

[1] Mean ±SEM of four replicate analyses of the number of pooled samples in parenthesis. [2] Values within a row superscripted by the same case letter are not significantly different at $P \leq 0.05$. Comparisons are made only within similar assay conditions, i.e. no added PLP or added PLP. [3] Rats representing each group were injected with 10 mg pyridoxine·HCl, fed the control diet ad libitum, and sacrificed at the times indicated after injection. Comparisons are made among 2 and 20 minute samples, among 24 hour samples, and among the 21 day repletion and 123 days of feeding the respective diets.

creased with the addition of PLP to levels significantly higher than that of R or C.

Within 2 minutes of pyridoxine injection in the repleted D rats the whole brain GAD activity increased to levels as high as or higher than that of similarly treated rats from R. Twenty minutes after injection of pyridoxine·HCl, whole brain GAD activity was significantly higher than that of R, with or without addition of PLP. Whole brain GAD activity without added PLP was essentially the same for all three dietary groups after 24 hours and 21 days of repletion. The whole brain GAD activity of the unrepleted rats from D was significantly lower than that of R or C after feeding the respective diets for 123 days. However, when PLP was added to the assay, the whole brain GAD activity of D was restored to levels similar to that of R, C

TABLE 3

The effect of vitamin B_6 deficiency on GAD activity in olfactory bulb of rats

Days fed diet	Deficient		Restricted		Control	
	No added PLP	Added PLP	No added PLP	Added PLP	No added PLP	Added PLP
0					591 ±71[1] (7)	809 ±23
5	507 ± 3[A,2] (3)	1,077 ±19[a]	915 ±22[B] (3)	1,344 ±31[b]	807 ±21[C] (3)	1,243 ±80[b]
8	672 ±57[A] (3)	1,076 ±18[a]	813 ±28[B] (3)	1,213 ±45[b]	896 ± 8[B] (3)	1,229 ±28[b]
17	440 ±21[A] (2)	1,035 ±17[a]	675 ±19[B] (2)	1,264 ± 5[b]	799 ±53[C] (2)	1,324 ±15[C]
102	412 ±34[A] (2)	823 ±16[a]	570 ±22[B] (2)	685 ±46[B]	522 ±22[B] (2)	667 ±47[a]
Repletion[3]						
2 min	550 ±11[A] (2)	1,078 ±21[a]	—	—	—	—
20 min	720 ± 9[B] (3)	1,088 ±32[a]	683 ±13[B] (2)	950 ±16[b]		
24 hrs	834 ±19[A] (3)	1,033 ±29[a]	703 ±57[A] (2)	948 ±13[b]	772 ±53[A] (2)	1,035 ±10[a]
21 days	812 ±70[A,B] (3)	1,067 ±10[a]	923 ±57[A] (3)	1,178 ±16[b]	683 ± 9[B] (3)	1,136 ±30[b]
123	430 ± 6[C] (3)	1,140 ±17[b]				

[1] Mean ±SEM of four replicate analyses of the number of pooled samples in parentheses. [2] Values within a row superscripted by the same case letter are not significantly different at $P \leq 0.05$. Comparisons are made only within similar assay conditions, i.e. no added PLP or added PLP. [3] Rats representing each group were injected with 10 mg pyridoxine·HCl, fed the control diet ad libitum, and sacrificed at the times indicated after injection. Comparisons are made among 2 and 20 minute samples, among 24 hour samples, and among the 21 day repletion and 123 days of feeding the respective diets.

TABLE 4

The effect of vitamin B_6 deficiency on olfactory bulb and whole brain weights

Dietary group	Tissue weight			
	Olfactory bulb		Whole brain	
	mg	*mg/100 g B.W.*	*g*	*g/100 g B.W.*
Deficient	91.6±11.4[a,1]	39.2±7.4[a]	1.677±0.148[a]	0.721±0.142[a]
Restricted	96.6±13.4[a]	31.4±4.8[b]	1.716±0.137[a]	0.558±0.066[b]
Control	96.1±14.4[a]	23.6±6.0[c]	1.791±0.142[a]	0.437±0.080[c]
Repleted[2]	108.7±17.8[a]	36.9±7.6[a,b]	1.723±0.101[a]	0.583±0.075[b]

[1] Mean±SD for eight to nine samples measured at the termination of the experiment (123 days). Values within a column with the same letter superscript are not significantly different at $P \leq 0.05$. [2] Rats were fed the pyridoxine deficient diet for 102 days, injected with 10 mg pyridoxine·HCl, and fed the control diet for 21 days.

and the repleted rats. This indicates that the levels of GAD protein in the whole brains of the vitamin B-6 deficient rats was unaffected by the deficiency and that the decrease in GAD activity observed when PLP was not added to the assay was due to depletion of the cofactor and not due to a decreased synthesis of GAD.

Olfactory bulb GAD activity (table 3) responded to the dietary pyridoxine deficiency in a manner similar to that of the whole brain. After 5, 8 and 17 days of feeding the vitamin B-6 deficient diet, the olfactory bulb GAD activity, with or without added PLP, was significantly lower than that of R and C. This was in contrast to the whole brain GAD activity which was consistently increased to levels the same as or higher than the control group values when PLP was added to the assay. Feeding the deficient diet for 102 and 121 days resulted in olfactory bulb GAD activities which were higher or the same as control activities when PLP was added to the assay medium.

Repletion of rats from D by injection of pyridoxine·HCl resulted in olfactory bulb GAD activities which increased after 20 minutes to a level similar to that of R, under the assay conditions of no PLP addition. Feeding the control diet to the injected D rats resulted in olfactory bulb GAD activities similar to those of the control groups (R and C), with or without added PLP. The rats representing D which were not repleted had olfactory bulb GAD activities significantly lower than R, C and the repleted rats when PLP was not added to the assay medium (table 3, 123 days).

The results of this study show that the GAD activity in the olfactory bulb and whole brain of rats is affected by dietary pyridoxine in a similar manner. Depletion of the cofactor, PLP, results in a decrease in the haloenzyme activity (no PLP added to GAD assay medium, tables 2 and 3). Addition of PLP to the GAD assay medium for both tissues always returned GAD activity to control levels which suggests that the synthesis of GAD protein was not affected by the deficiency and agrees with what has been reported for whole brain GAD (17). In some cases, there was a modest, although significant, increase in whole brain and olfactory bulb GAD activity in the deficient rats, relative to the controls, when PLP was added to the assay medium. This may represent a compensatory increase in the synthesis of GAD protein in both tissues of the deficient rats as was suggested by Bayoumi and Smith (18) for whole brain samples. GAD activity in both tissues also responded similarly to repletion, although parenteral administration of pyridoxine·HCl to deficient rats returned whole brain GAD activity to control levels at an apparently slightly faster rate than olfactory bulb GAD activity.

In some cases, AOAA (amino oxyacetic acid) was added to the GAD assay medium or to a portion of the tissue homogenate to insure that the $^{14}CO_2$ liberated during the assay was strictly due to GAD activity. AOAA is a carbonyl trapping agent which inhibits GAD activity in vitro (16). The addition of AOAA (1 μl of 80 μM AOAA; 1.6 μM AOAA final concentration in assay) to the GAD assay medium con-

taining added PLP prior to the initial incubation consistently inhibited GAD activity for both the olfactory bulb (e.g. 860 ± 19 versus 105 ± 7 pmole/mg protein/minute) and whole brain (e.g. 623 ± 3 versus 129 ± 5 pmole/mg protein/minute) samples. Addition of AOAA to a portion of the homogenate (1.28 mM AOAA in homogenate) also invariably resulted in inhibition of GAD activity (863 ± 47 versus 4 ± 3 pmole/mg protein/minute for olfactory bulb and 863 ± 47 versus 23 ± 23 pmole/mg protein/minute for whole brain).

Olfactory bulb and whole brain tissue weights were not affected by feeding the experimental diets unless expressed on a body weight basis (table 4). This may be interpreted to indicate that the decreased body weight of D and R were primarily due to a decreased accretion of muscle mass and adipose tissue while nervous tissue and perhaps other organs developed similar to that of C, on an absolute wet weight basis.

It would be interesting to determine if the in vivo decreases in olfactory bulb GAD activity have an influence on olfactory ability. It does not seem very likely that the decreased GAD activity would alter olfactory acuity in the adult rat since decreases of up to 75% in the carnosine content of the olfactory bulb have been shown to be without apparent effect (19). However, many of the recent vitamin B-6 deficiency studies have utilized pregnant dams and neonatal rats as models (20–23). Although the deficiency does not appear to affect the major constituents of the dam's milk, the concentration of pyridoxine in the milk is influenced by the level of dietary vitamin B-6 (21). There also appears to be a decrease in the amount of milk produced by the dams fed the pyridoxine deficient diet which has been suggested to be secondary to an inability of the pups to suckle (20). It has been demonstrated that olfactory cues in the rat are of paramount importance for nipple attachment during suckling, especially for the first several days of life, before vision and audition are functional (5, 6). The results from this study indicate that GAD activity in the rat olfactory bulb is affected by dietary vitamin B-6 in a manner which is similar to that of whole brain GAD. Since the olfactory bulbs of rats undergo postnatal synaptogenesis, it seems very likely that an altered olfactory ability may be an important factor which contributes to the inability of pyridoxine deficient neonatal rats to suckle.

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