Dietary Excess of Vitamin B-6 Affects the Concentrations of Amino Acids in the Caudate Nucleus and Serum and the Binding Properties of Serotonin Receptors in the Brain Cortex of Rats

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ABSTRACT Vitamin B-6 is a cofactor in many reactions of nitrogen metabolism. Deficiency alters tissue amino acid concentrations but effects of excess vitamin B-6 have not been well described. We fed female rats (218 g, 7 per group) 1 (control), 10, 100, 175 or 250X the National Research Council recommended level of pyridoxine HCl (7 mg/kg) for 10 wk and measured serum amino acids, amino acids and neurotransmitters in brain regions and the binding properties of serotonin receptors in the cerebral cortex using a ketanserin binding assay. Rats were decapitated, and unheparinized blood was obtained. In the caudate nucleus, concentrations of glutamate, threonine, taurine, methionine, γ-amino-butyric acid and the sum of the essential amino acids in groups 10X and 100X were ~130 to 180% of control levels (P < 0.05); groups 1X, 175X and 250X were not different. A similar pattern was seen in the serum for serine, glycine, aspartate and ornithine; the latter two amino acids increased to over 200% of control in group 100X. In the ketanserin binding assay, both the antagonist binding affinity and the maximal number of binding sites were higher for group 100X than for 1X, 175X and 250X, and were higher for 10X than for 1X. Norepinephrine in the raphe nucleus followed a similar biphasic pattern. Excess dietary pyridoxine affected brain and serum concentrations of some amino acids and binding properties of cortical serotonin receptors in a biphasic pattern over the range of concentrations fed in this study. J. Nutr. 128: 1829–1835, 1998.

KEY WORDS: vitamin B-6, amino acids, brain, blood, rats

The physiologically active forms of vitamin B-6 are enzymatic cofactors in many reactions of mammalian nitrogen metabolism, including the metabolism of most amino acids and neurotransmitters. Indeed, a dietary deficiency of vitamin B-6 affects tissue concentrations of amino acids and neurotransmitters in rats and humans (Dakshinamurti et al. 1988, Guilarte 1989, Park and Linkswiler 1971, Swenseid et al. 1964, Tews and Lovell 1967, Wasylnczuk et al. 1983a and 1983b). The patterns and directions of changes reported are somewhat variable, but in general, vitamin B-6 deficiency leads to a decrease in the concentrations of most amino acids. Swenseid et al. (1964) observed that the essential amino acids decreased more than the nonessentials in vitamin B-6 deficiency. Brain amino acids most commonly reported to be affected by deficiency are serotonin, dopamine and γ-amino-butyric acid (Dakshinamurti et al. 1988, Guilarte 1989).

It has been suggested that the central nervous system may be protected from excess vitamin B-6 by the blood-brain barrier and by the saturable transport mechanism for vitamin B-6, primarily because the toxicity syndrome associated with excessive consumption of vitamin B-6 is most obviously manifested in the peripheral nervous system (Schaumburg et al. 1983). Although there are few reports of the effects of excess vitamin B-6 on amino acid and neurotransmitter concentrations in the brain, some evidence shows that large doses of vitamin B-6 can affect central nervous system (CNS) function (Driskell and Loker 1976, Lee et al. 1988, Schaeffer 1993) and neurotransmitter, especially serotonin, concentration (Dakshinamurti et al. 1990). We decided, therefore, to measure concentrations of amino acids and neurotransmitters in brain regions of our rats; if the brain were protected from excess vitamin B-6, no changes would be expected.

We had designed an experiment in which adult rats were fed from 1 to 250X the National Research Council (NRC) recommended level of pyridoxine, and reported B-6 vitamer concentrations (Schaeffer et al. 1995) and changes in

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the startle response, a CNS reflex (Scheaffer 1993). We used the brains of some of those rats to examine the effects of a range of dietary vitamin B-6 concentrations on amino acid and neurotransmitter concentrations in several brain regions: the caudate nucleus and the cerebellum, because they are important in motor control and motor abnormalities have been observed with excess vitamin B-6 (Schaumburg et al. 1983); the raphe nucleus, because it is the site of serotonin cell bodies in the rest of the brain; the cortex, because it contains a high concentration of one class of serotonin receptors (serotonin_1D); the medial basal hypothalamus (MBH), because it is a major site of neuroendocrine control and previous work has shown an endocrine effect with administration of excess vitamin B-6 (Delitala et al. 1976).

Probably more than for any other CNS neurotransmitter, the kinetics of serotonin are sensitive to change in amino acid concentrations, partly because several other amino acids compete with its precursor, tryptophan, for transport into the brain. Because the B-6 vitamers differ in their concentrations and the binding properties of serotonin receptors in rat cortex have already been observed both in vitamin B-6 deficiency (Paulose and Dakshinamurti 1985) and with injection of pyridoxine (Dakshinamurti et al. 1990), we also measured the kinetic properties of the class of serotonin receptors called serotonin_2 in the cortex using the high affinity ligand [3H]-ketanserin.

Because of the relationship between brain and blood amino acid concentrations, we also measured serum amino acid concentrations.

MATERIALS AND METHODS

Animals and diets. The protocol for this experiment was approved by the animal use committees of the Western Human Nutrition Research Center and the Letterman Army Institute of Research (San Francisco). Sixty female 12-wk-old Long-Evans rats (Charles Rivers Laboratories, Wilmington, MA) were housed singly in stainless-steel hanging cages in a temperature-controlled room with a 12:12 h light:dark cycle. Rats were fed a commercial diet for 3 d and then a nutritionally complete purified diet (see below) for 7 d to allow acclimation to surroundings and diet before being assigned to experimental diets. Body weight at the time of diet assignment was 218 ± 14 g (mean ± SD). Rats were arranged into 12 groups of five rats with similar body weights (12 weight blocks) and assigned randomly to one of five dietary treatment groups (12 rats in each of five dietary groups in a fully randomized block design). The five diets (Dyets, Bethlehem, PA) varied only in the concentration of vitamin B-6 in the form of pyridoxine HCl, which replaced an equivalent amount of sucrose. By analysis, total vitamin B-6 concentrations of the diets expressed as mg of pyridoxine HCl/kg diet were 6, 65, 653, 1221 and 1773 by high performance liquid chromatography (HPLC) in our laboratory (Schaeffer et al. 1995). These levels represent ~1, 10, 100, 175 and 250X, respectively, the level recommended by the NRC (7 mg vitamin HCl/kg diet, NRC 1978); diets will be designated as 1X, 10X, 100X, 175X and 250X, respectively. The diets otherwise were formulated according to specifications of the American Institute of Nutrition (AIN) (AIN 1977 and 1980) and contained (g/100 g diet): casein, 20; sucrose, 50; corn-starch, 15; cellulose, 5.5; corn oil, 5.0; dl-methionine, 0.3; mineral mix, 3.5; vitamin mix, 1.0; choline bitartrate, 0.2; ethoxyquin, 0.01. Because no effect of 1230 mg of pyridoxine HCl/kg diet on food intake of rats of similar strain and age had been observed previously (Scheaffer et al. 1989), rats were not pair-fed but rather allowed free access to food. Food intake, corrected for spilled diet, was recorded daily and rats were weighed weekly using a computer-assisted weighing system (San Diego Instruments, La Jolla, CA). Fresh tap water was freely available.

Collection and analysis of tissue. After 10 wk of feeding, seven randomly chosen weight blocks (n = 7 for each diet group) were decapitated during the light cycle without anesthesia and without food deprivation to minimize the effects of stress on the variables measured. Unheparinized blood was obtained from the trunk; plasma was therefore not available from these rats. Rats were killed by weight block: order of kill was random among blocks and within blocks. Sera and brains were taken, frozen in liquid nitrogen and stored unexposed to light. Sera were analyzed for neurotransmitters after alumina extraction as previously described (Gietzen et al. 1991) and for amino acids by automated amino acid analysis after extraction with sulfosalicylic acid (Beckman 7300, Palo Alto CA). (Note that, because of extrusion or leakage from platelets and other cells, concentrations of amino acids in serum tend to be slightly higher than concentrations in plasma; this is true especially for taurine and glycine.)

Brains were dissected and samples of the caudate nucleus, MBH, cortex, cerebellum and raphe nucleus were prepared for analysis of neurotransmitters and amino acids as previously reported (Gietzen et al. 1986). Ketanserin binding studies were performed on cortex samples using a radioligand binding study adapted from Biegou et al. (1987). Briefly, whole cortices were dissected from frozen brains with a sample (15.1 ± 1.1 mg, mean ± SD) reserved for neurotransmitter and amino acid analysis. The remainder (543.2 ± 13.5 mg) was homogenized in Tris HCl buffer (50 mmol/L, pH 7.4) using a Polytron (setting 8 for 45 s; Brinkmann Instruments, Westbury, NY), and protein concentrations were determined using a modification of the Lowry technique (Markwell et al. 1978). Samples were brought to 1 g protein/L by addition of Tris HCl buffer. A seven-point saturation binding isotherm was generated from each cortex by incubating cortex homogenates with [3H]-ketanserin HCl (2.4 TBq/mol, New England Nuclear, Boston, MA). Homogenate (150 μL) was incubated with 50 μL of incubation buffer (50 mmol/L of Tris-HCl, 100 mmol/L of MgCl_2, or 10 μmol/L of mianserin HCl (Research Biochemicals Int., Natick, MA) to define nonspecific binding, and 50 μL of differing concentrations of [3H]-ketanserin (0.65–3.0 mmol/L final concentration) in capped 12 × 75 mm polypropylene tubes. The incubation was done for 60 min in the dark at room temperature (25°C). The reaction was stopped by washing 9–10 times with cold buffer (50 mmol/L of Tris-Acetate) in a 24-manifold cell harvester (Brandel, Gaithersburg, MD) using dry Whatman GF/B filters (Maidstone, England) previously soaked in 10 g/L of bovine serum albumin. Radioactivity remaining on filters was determined by scintillation counting (Packard, Palo Alto, CA). Specific binding was determined by subtracting radioactive in the presence of mianserin from total radioactivity. A Rosenthal (1967) analysis for each assay was done by computerized linear regression analysis to estimate total binding site density (B_max in fmol bound/mg protein) and receptor affinity (K_d in μmol/L).

The remaining five weight blocks were used to determine concentrations of B-6 vitamers in plasma and tissues after overnight food deprivation; procedures (HPLC) and results were reported previously (Scheaffer et al. 1995). Results from whole brains and plasma will be presented in this report because of their relevance to the current study.

Statistical methods. Data were analyzed with the SAS System for Personal Computers, version 6.04 (SAS Institute, Cary, NC). The analysis of variance model for all variables, including body weight and average daily food intake, contained factors for weight block and diet. (Inclusion of other factors in the model was not possible because of the relatively small number of animals in each group.) If the main effect of diet was significant, comparisons of least squares means were performed. Where the usual significance level for rejection of outliers is α = 0.10, we used a more rigorous rejection criterion of α = 0.02.
RESULTS

Dietary pyridoxine concentration did not significantly affect final body weight or average food intake of rats, although there was a trend for lower food intake with higher pyridoxine concentrations (P = 0.06) (Table 1). At no time were overt toxicity symptoms observed in any group.

Concentrations of the principal B-6 vitameric forms in whole brains and plasma are shown in Table 2. There were no significant effects in brain of dietary pyridoxine concentration on concentrations of either pyridoxal phosphate or pyridoxamine phosphate, the active coenzyme forms of vitamin B-6. In plasma, the concentration of pyridoxal, the principal transport form of vitamin B-6, increased significantly with increases in dietary pyridoxine, but there was no effect on plasma pyridoxal phosphate concentration.

Because of the wide range (typical in biological samples) in mean concentrations among the different amino acids, results of amino acid analyses are shown as percentage of the control group (group 1X). However, all statistical analyses were done with raw data rather than percentages; the mean ± SEM (μmol/L) for the control group is given in the figure legends.

In the caudate nucleus, concentrations of a number of amino acids were significantly affected P < 0.05 by dietary pyridoxine HCl concentration (Fig. 1). Concentrations of glutamate, threonine, taurine, methionine, γ-amino-butyric acid and the sum of the essential amino acids (phenylalanine, valine, threonine, methionine, isoleucine, leucine, lysine and tyrosine) in groups 10X and 100X were ~130 to 180% of control levels, and, with the exception of methionine, were not significantly different between the two groups; groups 1X, 10X, 175X and 250X were not significantly different from each other. This inverted U-shaped dose-response pattern could be seen with other amino acids in the caudate nucleus, with concentrations reaching up to 150% of control in group 100X; however, the effect of dietary pyridoxine on those amino acids was not significant and is not depicted: alanine, lysine (0.05 < P < 0.1); serine, aspartate, total amino acids (0.1 < P < 0.2); and glycine, valine, isoleucine, tyrosine and phenylalanine (0.2 < P < 0.3). No significant effect of dietary pyridoxine on amino acid concentrations in the MBH, cortex, cerebellum and raphe nucleus was seen and no pattern of response was evident in those areas (data not shown).

A biphasic response pattern, similar to that seen in the caudate, was seen for several serum amino acids (Fig. 2); serine, glycine, aspartate and ornithine were significantly affected by dietary pyridoxine (P < 0.05); aspartate and ornithine increased to over 200% of control in group 100X. The effect of dietary pyridoxine on total amino acids (P = 0.09) and phenylalanine (P = 0.06) was similar in pattern. A few other amino acids responded similarly, but these amino acids increased to a maximum of only about 115% of control, and the effect of dietary pyridoxine was not significant: glycine (0.1 < P < 0.2) histidine, threonine and the sum of the essential amino acids (0.2 < P < 0.3).

Results of the ketanserin binding assay are shown in Figure 3. The parameters, βMax and KD, were derived from Rosenthal plots having a mean r² (± SEM) of 0.83 (± 0.02; n = 31), with a range of 0.48–0.99. The same biphasic pattern was evident in both response parameters. The response of diet group 100X was significantly higher than that of 1X, 175X and

### TABLE 1

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>1X</th>
<th>10X</th>
<th>100X</th>
<th>175X</th>
<th>250X</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>277</td>
<td>269</td>
<td>269</td>
<td>265</td>
<td>264</td>
<td>4.3</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>11.7</td>
<td>11.4</td>
<td>11.2</td>
<td>10.8</td>
<td>11.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1 Values are means, n = 12. Diet 1X contained ~7 mg of pyridoxine HCl/kg diet; other diets contained the indicated multiples of 7 mg of pyridoxine HCl/kg. Effect of diet in analysis of variance for body weight was P = 0.25; for food intake, P = 0.06. These data have been reported previously (Schaeffer et al. 1995).

### TABLE 2

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>1X</th>
<th>10X</th>
<th>100X</th>
<th>175X</th>
<th>250X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxal phosphate</td>
<td>786 ± 70</td>
<td>826 ± 22</td>
<td>691 ± 89</td>
<td>655 ± 109</td>
<td>791 ± 153</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>1032 ± 96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1141 ± 87&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1737 ± 284&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2189 ± 626&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2570 ± 588&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain, nmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxal phosphate</td>
<td>6.7 ± 0.3</td>
<td>6.7 ± 0.3</td>
<td>6.6 ± 0.5</td>
<td>6.4 ± 0.5</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>Pyridoxamine phosphate</td>
<td>9.8 ± 0.3</td>
<td>10.0 ± 0.3</td>
<td>9.7 ± 0.4</td>
<td>9.0 ± 0.2</td>
<td>9.9 ± 0.4</td>
</tr>
</tbody>
</table>

1 Values were determined by high performance liquid chromatography and were reported previously (Schaeffer et al. 1995). Values are means ± SEM, n = 5, except for plasma of 1X in which n = 4. Diet 1X contained ~7 mg of pyridoxine HCl/kg diet; other diets contained the indicated multiples of 7 mg of pyridoxine HCl/kg. Rats were deprived of food overnight. The data were first tested for heterogeneity of variance with Bartlett’s test; only plasma pyridoxal concentrations required transformation before analysis of variance. Errors were not pooled because of the heterogeneity of variance in plasma pyridoxal concentrations. Within a row, values with different superscripts are significantly different (P < 0.05) by the protected least significant difference test.
Concentrations of some amino acids in the serum of adult female rats fed excess pyridoxine. Results are shown as percentage of control (group 1X); mean concentration ± SEM for group 1X (nmol/mg) of each amino acid is as follows: Glu = 7.06 ± 0.35, Thr = 0.70 ± 0.07, Tau = 4.1 ± 0.39, Met = 0.026 ± 0.002, γ-amino-butyric acid (GABA) = 2.02 ± 0.29, essential amino acids (EAA) = 1.14 ± 0.1, n = 7. Diet 1X contained −7 mg of pyridoxine HCl/kg diet; other diets contained the indicated multiples of 7 mg of pyridoxine HCl/kg. For each amino acid, the significance level for the main effect of diet in the analysis of variance is shown on the figure; bars with different superscripts are significantly different (P < 0.05) by the protected least significant difference test.

We observed a striking biphasic response to increasing levels of excess dietary pyridoxine for some amino acids in one brain area, the caudate nucleus, and in the serum; concentrations were elevated in rats fed diets containing 10X and 100X the NRC recommended level (NRC 1978) and were near or below control levels in groups fed 175X and 250X the NRC. Total amino acids (the sum of all amino acids measured) followed the same pattern in the caudate and serum, but the effect of diet was not significant (P = 0.09 and P = 0.15, respectively). A greater number of amino acids was affected by dietary pyridoxine in the caudate than in the serum, but none of the same amino acids was affected in both tissues. In the caudate, the essential amino acids were affected, along with glutamate, taurine and γ-amino-butyric acid, which are neuronally active; in serum, only nonessential amino acids were significantly affected.

Brain amino acid concentrations are controlled by selective transport mechanisms at the blood-brain barrier and by specific metabolizing enzymes within the tissue. In general, brain metabolites in some brain sections are presented in Table 3. The only significant effect of diet was on norepinephrine concentration in the raphe nucleus: it was significantly lower in the group fed the greatest concentration of pyridoxine (250X) than in groups 10X–175X but was not significantly different from that of group 1X, revealing a biphasic pattern similar to those observed in other variables described above. There were no other significant effects of diet on brain regional neurotransmitter concentrations, although there was a suggestion of a biphasic response pattern in some variables in the cerebellum and caudate; that is, concentration tended to be highest in group 100X; see, for example, 3-methoxy-4-hydroxy-phenylglycol in the cerebellum (P < 0.2).

FIGURE 1 Concentrations of the amino acids significantly affected by diet in the caudate nucleus of adult female rats fed excess pyridoxine. Results are shown as percent of control (group 1X); mean concentration ± SEM for group 1X (nmol/mg) of each amino acid is as follows: Glu = 7.06 ± 0.35, Thr = 0.70 ± 0.07, Tau = 4.1 ± 0.39, Met = 0.026 ± 0.002, γ-amino-butyric acid (GABA) = 2.02 ± 0.29, essential amino acids (EAA) = 1.14 ± 0.1, n = 7. Diet 1X contained −7 mg of pyridoxine HCl/kg diet; other diets contained the indicated multiples of 7 mg of pyridoxine HCl/kg. For each amino acid, the significance level for the main effect of diet in the analysis of variance is shown on the figure; bars with different superscripts are significantly different (P < 0.05) by the protected least significant difference test.
concentrations of amino acids, especially the nonessentials, are not easily influenced by dietary composition. Blood amino acid concentrations, on the other hand, primarily reflect dietary composition but also reflect changes in amino acid metabolism in many tissues throughout the body. We did not measure concentrations of amino acids in any other tissue, so whether a pattern is reflected elsewhere is unknown.

The changes in amino acid concentrations we observed occurred without concomitant changes in the same tissues of the concentrations of pyridoxal phosphate and pyridoxamine phosphate, the coenzymatic forms of vitamin B-6 involved in amino acid metabolism. This is consistent with our previously reported results (Schaeffer et al. 1989) and the reports of others (Bender and Totoe 1984, Cohen et al. 1973, Lee et al. 1988).

It is intriguing that the caudate nucleus was the only area of the brain in which changes in amino acid concentrations were observed. The caudate nucleus has an important role in the control of movement, and abnormalities of motor coordination are a principal expression of pyridoxine toxicity. While motor abnormalities associated with pyridoxine toxicity are thought to be primarily peripheral in origin (Anonymous 1984), involvement of the CNS is certainly conceivable. Pyridoxine toxicity, with its associated weight loss and gait changes, was not induced in this experiment; moreover, as in a previous study (Schaeffer et al. 1989), neurotransmitter concentrations within the caudate nucleus were not affected by the level of pyridoxine fed. It would be interesting to determine neurotransmitter and amino acid concentrations in brain regions of rats with overt vitamin B-6 toxicity and motor abnormalities.

Other investigators have described various effects of excess dietary vitamin B-6 on amino acid concentrations and metabolism in rats. Cohen et al. (1973) fed young rats either 50 mg (control) or 500 mg of pyridoxine HCl/kg diet for 5 wk then deprived them of food for 24 h before decapitation; they found no difference between groups in plasma and tissue (including whole brain) amino acid concentrations. In our study, concentrations of serum amino acids in the two groups fed diets most closely approximating theirs (10 and 100X) were similar; were these the only diets fed, the two groups probably could not have been distinguished statistically. Had Cohen et al. used a lower pyridoxine concentration as their control, they may have indeed found an effect of pyridoxine on some plasma amino acids. Khairallah and Moore (1966) found evidence of increased amino acid catabolism in rats fed 2000 mg of pyridoxine (HCl)/kg diet, including increased activities of several hepatic pyridoxal phosphate-dependent amino acid metabolizing enzymes. (The vitameric form used was not specified.) Likewise, the activities of several hepatic enzymes increased with injection of large doses of pyridoxine HCl (1000 mg/kg) in adult male rats (Greengard and Gordon 1963; Holten et al. 1967) and hepatic amino acid pools were altered (Holten et al. 1967). Following injection of pyridoxine HCl (10 mg/kg) to adult female rats, hepatic tryptophan catabolism decreased and brain and plasma tryptophan concentrations increased; plasma concentration of total amino acids was unaffected (Bender and Totoe 1984).

Chronic oral administration of large-doses of pyridoxine may increase amino acid metabolism in humans as reflected in plasma amino acid concentrations. Concentrations of aspartate, glutamine, methionine, isoleucine, tyrosine, total essential amino acids, total amino acids, the sum of the branched-chain amino acids and the sum of the aromatic amino acids were increased significantly in the plasma of fasting women 7 d after supplementation with 25 mg of pyridoxine HCl/d but had returned to control levels by 14 d. Phosphoserine, alanine, cysteine, arginine (all nonessential), urea, and pyridoxal phosphate concentrations were significantly elevated and glutamate concentration was decreased at both 7 and 14 d (Kang-Yoon and Kirksey 1992). In a different study, however, only valine concentration was elevated in plasma of fasting healthy

### TABLE 3

Concentrations of the principal neurotransmitters and metabolites in selected brain regions of adult female rats fed excess pyridoxine

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>1X</th>
<th>10X</th>
<th>100X</th>
<th>175X</th>
<th>250X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex, nmol/g</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>985±161</td>
<td>1226±436</td>
<td>1012±161</td>
<td>754±265</td>
<td>1099±228</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>310±46</td>
<td>349±68</td>
<td>302±63</td>
<td>436±131</td>
<td>312±33</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>524±46</td>
<td>443±72</td>
<td>516±75</td>
<td>554±124</td>
<td>431±59</td>
</tr>
<tr>
<td>Raphe nuclei, nmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>2765±784</td>
<td>2004±678</td>
<td>1805±369</td>
<td>2999±604</td>
<td>1292±233</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>2191±876</td>
<td>1784±402</td>
<td>1639±382</td>
<td>2054±450</td>
<td>1571±155</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1718±319ab</td>
<td>2103±154a</td>
<td>1882±284a</td>
<td>2189±246a</td>
<td>1149±143b</td>
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<td>Caudate nucleus, nmol/g</td>
<td></td>
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<tr>
<td>Dopamine</td>
<td>1477±282</td>
<td>2099±302</td>
<td>2616±452</td>
<td>2185±419</td>
<td>2136±206</td>
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<tr>
<td>5-HIAA</td>
<td>462±75</td>
<td>491±70</td>
<td>485±82</td>
<td>349±66</td>
<td>508±51</td>
</tr>
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<td>Cerebellum, nmol/g</td>
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<td>MHPG</td>
<td>289±45</td>
<td>353±31</td>
<td>594±200</td>
<td>370±131</td>
<td>208±39</td>
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<tr>
<td>Kynurenine</td>
<td>196±78</td>
<td>103±39</td>
<td>117±55</td>
<td>281±108</td>
<td>108±46</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>467±88</td>
<td>415±22</td>
<td>505±113</td>
<td>480±89</td>
<td>309±22</td>
</tr>
<tr>
<td>DOPAC</td>
<td>252±114</td>
<td>297±85</td>
<td>380±110</td>
<td>219±83</td>
<td>159±35</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 4–7. 5-HIAA = 5-hydroxyindoleacetic acid; DOPAC = dihydroxyphenylacetic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol. Diet 1X contained −7 mg of pyridoxine HCl/kg diet; other diets contained the indicated multiples of 7 mg of pyridoxine HCl/kg. Within a row, values with different superscripts are significantly different (P < 0.05) by the protected least significant difference test.

2 One positive outlier was rejected from group 100X using Dixons test for statistical outliers (Dunn and Clark 1974).
adults after 2 wk of 300 mg of pyridoxine HCl/d (Kleiner et al. 1980).

In general, then, investigators have found an increase in tissue concentrations of some amino acids or no change with administration of large doses of pyridoxine HCl. Such effects could be mediated by changes in intestinal absorption, transport among tissues or metabolism of amino acids. Williams (1964) reviewed evidence both supporting and contradicting the possibility that excess vitamin B-6 may stimulate amino acid transport; such effects may exist for some amino acids and not for others. A stimulation of intestinal absorption of amino acids by vitamin B-6 seems unlikely since amino acids are absorbed as quickly as they are released in proteolysis. Transport among tissues could be affected: Demisch and Kaczmarczyk (1991) suggested that supplemental vitamin B-6 might stimulate an increase in tryptophan transport into serotonergic neurons, inducing an increase in serotonin synthesis. It has also been suggested that some vitamin B-6-dependent enzymes are unsaturated at physiological conditions and that their activity is enhanced by administration of pyridoxine (Ebadi et al. 1973). Effects of excess vitamin B-6 on amino acid metabolism could also be mediated through effects on hormonal balance. Indeed, a single dose of pyridoxine (300 mg i.v.) produced a significant increase in growth hormone and a decrease in prolactin in plasma of humans (Delitala et al. 1976). Growth hormone affects amino acid metabolism and protein synthesis. Holten et al. (1967) attributed an increase in activity of rat hepatic tyrosine transaminase with pyridoxine injection to an effect on hormonal balance, probably that of growth hormone. Interestingly, the amino acid transport defects seen in vitamin B-6 deficiency may be associated with decreased levels of growth hormone (Heindel and Rigs 1968).

Neurotransmitter concentrations in brain regions were, for the most part, not affected by diet in our study. Similarly, Lee et al. (1988) reported that hypothalamic concentrations of serotonin and its metabolite, 5-hydroxyindoleacetic acid, were not affected in male weanling rats after 10 d of consuming a diet containing 3000 mg of pyridoxine HCl/kg; however, when excess tryptophan was included in the diet, an increase in hypothalamic serotonin was observed. Bender and Toteo (1984) also reported that whole brain concentration of serotonin in adult female rats was not affected following an i.p. injection of 10 mg of pyridoxine HCl/kg body weight. Interestingly, they observed an increase in both the concentration of tryptophan, the precursor of serotonin, and in serotonin turnover as indicated by a significant increase in radioactivity in serotonin following an i.p. injection of 3H-tryptophan. Therefore, a lack of change in neurotransmitter concentrations with pyridoxine administration, as was found in ours and others’ studies cited above, does not exclude the possibility of an effect on neurotransmitter metabolism.

In contrast to the above observations, Dakshinamurti et al. (1990) reported that the concentration of serotonin in most brain regions, including the cortex, hypothalamus and cerebellum, was significantly elevated in adult rats (sex unspecified) injected i.p. with 100 mg of pyridoxine (HCl)?/kg for 7 d. Assuming the vitamer form given in that study was pyridoxine HCl, the dose was approximately equivalent to consumption of a 1000 mg/kg diet by our calculation. Interestingly, when injected i.p. to adult food-deprived rats, the several vitameric forms of vitamin B-6 had different effects on concentrations of plasma and adrenal catecholamines, the synthesis of which from vitamin B-6-dependent enzymes (Lau-Cam et al. 1991). Important factors affecting results in all the above studies could be the vitamer form administered, the concentration of vitamin B-6 given, the duration and route of administration, other components in the diet and the sex, fed/starved state and age of the rats.

While determining the concentration of a neurotransmitter in a tissue is important for understanding the impact of diet on that system, measuring the number or sensitivity of neurotransmitter receptors is also important. For example, both the concentration of serotonin and the kinetic parameters of its receptors were reported to be altered in the cerebral cortex of 21-d-old rats born of vitamin B-6-deficient dams (Paulose and Dakshinamurti 1985). One class of serotonin binding sites (serotonin1) is found in high concentration in the prefrontal cortex of the rat. Ketanserin is a high affinity ligand for those sites and can be used to determine their kinetic parameters: βmax, the maximal number or density of binding sites (fmol/mg protein), and Kd, the apparent binding affinity (nmol/L). βmax is directly related to receptor concentration in the tissue (sensitivity) and can be up or down regulated in response to ligand activity; e.g., decreased activity of the transmitter at the receptor can cause upregulation of the receptor and thus increase βmax; the reverse also applies. Kd on the other hand, is less likely to be affected by transmitter activity. Affinity is related to how long the ligand stays on the receptor and how tightly it is bound. Because these are G-protein-coupled receptors, protein interactions within the cell, as part of innumerable signal transduction mechanisms, likely play a role in affecting the magnitude of Kd.

While excess dietary pyridoxine did not affect cortical serotonin concentrations in our study, there was a biphasic response, in the same direction, in both kinetic parameters of the serotonin1 receptor: both parameters for groups 10X and 100X were significantly greater than those of the controls; those for 175X and 250X were not different from controls. While it is more common to see changes in βmax than in Kd in an increase in both parameters as seen in groups 10X and 100X, it is consistent with a higher population of lower affinity receptors (at least where the number of available receptors is a limiting factor). Perhaps the parallel changes in βmax and Kd are secondary to some common changes in the intracellular G-proteins or other signal transducing agents that are associated with the receptor. As was noted above, a lack of change in the concentration of a neurotransmitter does not necessarily mean that there has been no effect on its metabolism or function.

In contrast to our results, when Dakshinamurti et al. (1990) injected adult rats i.p. with 100 mg of pyridoxine (HCl)/kg for 7 d, βmax was significantly lower than in controls and Kd did not differ from controls in all brain regions studied; serotonin concentration was significantly higher in most brain regions. By our calculation, their dosage protocol approximated the pyridoxine intake of the rats in our 175X group, in which βmax was slightly, though not significantly, higher than controls and Kd did not differ from controls.

The route and duration of administration of pyridoxine differed markedly between our study and the work of Dakshinamurti et al. (1990). Moreover, the sex of the rats used in the two studies may be different: we used female rats and the sex of the rats used by Dakshinamurti et al. (1990) was not reported. Gonadal steroids have a significant role in the regulation of serotonin activity as expressed in receptor site kinetic parameters (Bieg 1990, Bieg et al. 1987). Both of these factors make comparison of results difficult.

We observed a biphasic response of a number of variables over the range of dietary vitamin B-6 used in this study. Other results indicate that physiologic or biochemical responses to the degree of vitamin B-6 nutrient are not necessarily linear over time. Lal and Dakshinamurti (1993) showed a biphasic
response of systolic blood pressure to decreasing vitamin B-6 nutriture in rats, such that blood pressure rose from wk 4 to wk 9 of deficiency, then decreased to control levels by wk 12. Acoustic startle response was lower than in controls in adult female rats fed excess dietary pyridoxine HCl for 7 wk (Schaef- fer 1993). It was greater than in controls after 3 wk of vitamin B-6 deficiency (Schaef et al. 1988) but in a separate study, lower at wk 17 of deficiency (Schaef 1987).

Our results suggest alterations of amino acid metabolism and of the kinetics of serotonin receptor sites, which are secondary to prolonged intake of moderate but not extreme excesses of vitamin B-6. Investigation into possible mechanisms productive of this pattern is warranted.

Only by feeding a wide range of dietary concentrations were we able to observe the biphasic pattern of response. For example, if we had used a single diet containing 250X the NRC recommended level, we might have concluded that excess vitamin B-6 has no effect on, or perhaps even results in a decrease in, the concentrations of some amino acids or in the serotonin receptor binding parameters measured. It is clear that “excess vitamin B-6” is not a single phenomenon; i.e., the degree of excess over the requirement is one among many variables critical in determining even the direction of re-

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