Quantitative Studies on Tryptophan Metabolism in the Pyridoxine-deficient Rat

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ABSTRACT Female rats weighing about 40 g were fed a pyridoxine-deficient diet. Control animals received the same diet supplemented with 2.5 mg of pyridoxine hydrochloride/kg of diet. After 2 or 3 weeks the animals were given 40 mg of L-tryptophan by the intraperitoneal route. Urine collected for 24 hours before and after the supplementation with tryptophan was analyzed for several tryptophan metabolites. The pyridoxine-deficient rats excreted abnormally large quantities of kynurenine, 3-hydroxykynurenine, acetylpyruvic acid and xanthurenic acid as compared with the control rats. The deficient animals and the controls excreted similar quantities of indoxyl sulfate, anthranilic acid glucuronide, o-aminobipuric acid and kynurenic acid. Although precursors of both xanthurenic and kynurenic acids were increased in the urine of the pyridoxine-deficient animals after a loading dose of tryptophan, kynurenic acid excretion was no greater than that of the control animals. These results are consistent with the view that the enzyme system concerned with the transamination of kynurenine to form kynurenic acid is distinct from the enzyme system which transaminates hydroxkynurenine to form xanthurenic acid. It is also possible that there is an alternate pathway for xanthurenic acid synthesis which remains active in pyridoxine deficiency.

Abnormal tryptophan metabolism similar to that noted in a pyridoxine deficiency state has been observed in a number of clinical conditions (1). Wachtstein and Lobel (2) demonstrated that normal pregnant women exhibited increased urinary excretion of tryptophan metabolites, and these authors commented on the similarity of paper chromatograms of urine from pregnant women and urine from pyridoxine-deficient rats. Brown et al. (3) confirmed and extended the above studies in pregnant women, using quantitative assays of urinary metabolites. They found that after a loading dose of tryptophan, normal pregnant women excreted elevated urinary levels of kynurenine, N-acetylkynurenine, 3-hydroxykynurenine, xanthurenic acid and N-methyl-2-pyridone-5-carboxamide (pyridone). Following administration of pyridoxine hydrochloride to these women, the metabolism returned to a more normal pattern, but the excretion of kynurenine, pyridone, and hydroxykynurenine remained somewhat elevated.

It was previously shown (4) that patients receiving pyridoxine antagonists had a pattern of tryptophan metabolism that in some respects resembled the pattern observed in pregnancy (3). The administration of pyridoxine to these patients, however, was associated with a return of the metabolism to normal. Since pyridoxine failed to completely restore the metabolism of pregnant patients to normal, it was suggested (3) that in addition to a functional pyridoxine deficiency, altered hormonal control of enzyme activities was probably a factor in the abnormal tryptophan metabolism in pregnancy.

The above speculation receives support from studies by Mason and Gullekson (5) concerning the effects of estrogen conjugates on the reactivation of resolved kynurenine transaminase. Furthermore, Porter and associates (6) showed that adrenalectomized rats excreted extremely low levels of kynurenic acid following a loading dose of tryptophan even though xanthurenic acid excretion was normal.

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Administration of cortisone to these rats preceding the tryptophan load resulted in the excretion of normal quantities of both kynurenine and xanthurenic acids.

Consequently, it seemed important to study in more detail the effects of pyridoxine deficiency per se on tryptophan metabolism in the rat. Dalgleish (7) studied tryptophan metabolism in pyridoxine-deficient rats, but the assay procedures were qualitative and semiquantitative in nature. Furthermore, Boone et al. (8) measured xanthurenic acid excretion and Porter et al. (9) determined kynurenine, kynurenic acid and xanthurenic acid in the urine of pyridoxine-deficient rats. A more complete quantitative study, however, was not available. Since the clinical studies conducted in these laboratories entail the measurement of 9 or more urinary metabolites of tryptophan, it was decided to measure these same metabolites in the urine of pyridoxine-deficient rats, using the same quantitative procedures as in the clinical studies.

MATERIALS AND METHODS

Animals and diet. Female albino rats weighing 40 to 45 g each were purchased from the Holtzman Company of Madison, Wisconsin. The rats were housed in steel-wire pens in groups of 5 and were fed ad libitum.

A purified diet was prepared which contained no added pyridoxine (table 1). This pyridoxine-deficient diet was converted to a control diet by the addition of 2.5 mg of pyridoxine hydrochloride/kg of diet.

**Urine collections.** Five rats were fed the pyridoxine-deficient diet and 5 rats were maintained with the control diet for 14 days. The animals were then placed in stainless steel metabolism cages in groups of 5 and a basal 24-hour pooled urine sample was obtained for each group. Each rat was then given an intraperitoneal injection of 40 mg of L-tryptophan in aqueous solution, and a second 24-hour pooled urine sample was obtained from each group. The rats then continued to be fed their respective diets for one additional week, after which time they were returned to the metabolism cages and the urine collections were carried out as outlined above prior to and after a second intraperitoneal injection of 40 mg of L-tryptophan.

**Analytical methods.** Indoxyl sulfate, anthranilic acid gluturionate, o-aminophipuric acid, acetyl-kynurenine, anthranilic acid and kynurenine were determined by the previously reported methods of Brown and Price (10). Hydroxykynurenine was determined using the procedure of Brown (11), and analyses for kynurenine and xanthurenic acid were performed according to the procedure of Satoh and Price (12).

N-Methyl-2-pyridine-5-carboxamide was not determined since an earlier study by Brown and Price (10) had shown it to be a trace metabolite in rats.

**RESULTS**

The urinary excretion levels of the various metabolites of tryptophan (table 2) represent data obtained from two separate experiments, in each of which urine collections were made after feeding the respective diets for 2 and 3 weeks. Since there was no apparent difference in the metabolic pattern at 2 weeks as compared with that at 3 weeks, these observations were combined. Thus, the values in table 2 represent the averages of these 4 separate determinations.

The pyridoxine-deficient rats excreted abnormally large quantities of kynurenine, hydroxykynurenine, and xanthurenic acid and moderately elevated levels of acetyl-
kynurenine as compared with the control rats. Kynurenic acid excretion by the pyridoxine-deficient rats was of the same magnitude as that of the control rats.

During the course of the experiments, the rats receiving the control diet had an average weight gain of 59 g/rat/3-week period. The rats fed the pyridoxine-deficient diet had an average weight gain of 25 g/rat/3-week period.

DISCUSSION

The results of these studies corroborate the earlier observations by Dalgleish (7) and by Wachstein and Lobel (2) of abnormal urinary levels of tryptophan metabolites in pyridoxine-deficient rats and demonstrate that the excretory pattern of tryptophan metabolites in these animals is qualitatively and quantitatively similar to that in the urine of normal pregnant women not receiving supplemental pyridoxine (3).

The magnitude of the increase in kynurenic acid excretion by the pyridoxine-deficient rats following tryptophan supplementation was essentially identical to that of the control animals (table 2). It was previously noted that kynurenic acid excretion by pregnant human females given tryptophan supplements was not significantly different from that of nonpregnant females, whereas xanthurenic acid excretion was elevated. This prompted the speculation that kynurenic transaminase and hydroxykynurenic transaminase may be distinct enzymes which respond differently to the hormonal stimuli of pregnancy. The studies of Porter et al. (6) demonstrate clearly that cortisone had markedly different effects on the in vivo synthesis of kynurenic acid and xanthurenic acid in the rat. It appears, therefore, that both hormonal factors and pyridoxine deficiency may have different effects on the biosynthesis of these two acids. Snell (13) pointed out that various pyridoxine-requiring enzymes exhibit a differential sensitivity to pyridoxine deficiency. It may be, therefore, that hydroxykynurenic transaminase is less sensitive to pyridoxine deficiency than is kynurenic transaminase. Thus, in the presence of an accumulation of comparable quantities of kynurenic and hydroxykynurenic acid, only xanthurenic acid excretion increased, which suggests that hydroxykynurenic transaminase retained high capacity to utilize substrate whereas kynurenic transaminase had suffered from the deficiency of coenzyme.

These results tend to support the suggestion by Brown et al. (3) that the transamination of kynurenic and hydroxykynurenic may involve 2 distinct enzymes with dissimilar characteristics. Another possibility is that an additional pathway for xanthurenic acid synthesis exists which does not utilize pyridoxine-linked coenzymes. Knox (14) has suggested that
oxidative deamination may play a role in this metabolic scheme, and Charonnet-Harding et al. (15) have demonstrated that xanthurenic acid excretion was increased in riboflavin-deficient rats. Henderson and co-workers (16) showed that riboflavin-deficient rats excreted elevated levels of xanthurenic acid following the administration of L-tryptophan but not following the administration of kynurenine or 3-hydroxykynurenine. The latter data support the theory that some alternate pathway for xanthurenic acid synthesis exists.

Ogasawara et al. (17) explained the increased formation of xanthurenic acid in pyridoxine-deficient rats on the basis of a decreased conversion of kynurenine to anthranilic acid and to a lesser extent, a decrease in its conversion to kynurenic acid. This would result in an accumulation of 3-hydroxykynurenine, which is not readily metabolized to 3-hydroxyanthranilic acid, but rather is converted to xanthurenic acid by mitochondrial kynurenine transaminase, which is not impaired extensively by pyridoxine deficiency. However, the results in Table 2 show that the precursor of kynurenine acid (kynurenine) accumulated in pyridoxine-deficient animals to levels comparable to the levels of the immediate precursor of xanthurenic acid (3-hydroxykynurenine). Thus, the mechanism suggested by Ogasawara et al. (17) does not account for the failure to observe an increase in the conversion of tryptophan to kynurenine acid in pyridoxine deficiency unless one assumes either that kynurenine transaminase suffers more as a result of the deficiency than hydroxykynurenine transaminase or that there is an alternate pathway to xanthurenic acid which may not involve pyridoxine.

The prior demonstration that administration of pyridoxine to pregnant women was associated with an improvement in tryptophan metabolism was suggestive evidence that pyridoxine deficiency may exist in pregnancy. Furthermore, Willis et al. (18) have reported the successful use of pyridoxine in the therapy of hyperemesis gravidarum, an extremely severe form of nausea and vomiting observed occasionally in pregnancy. In the past 2 decades obstetricians have reported success in the use of pyridoxine in the treatment of nausea of pregnancy (19, 20).

The data obtained in the present studies indicate that pyridoxine deficiency per se may play a role in the abnormal manner in which tryptophan is metabolized by pregnant women. Whether or not there is a correlation between these biochemical abnormalities and the clinical observations of Willis et al. (18) and others is still open to speculation.

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