The Role of Picolinic Acid in Metal Metabolism

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ENDOGENOUS SYNTHESIS OF PICOLINIC ACID

The synthesis of picolinic acid and the enzyme responsible for its production in mammalian systems were first described by Mehler in 1956. In this early experiment, Mehler demonstrated that acetone powders of rat liver metabolized 3-hydroxyanthranilic acid in two steps. In the first step, the substrate was oxidized to form a compound that had an absorbance maximum at 360 nm. Mehler discovered that this intermediate was converted non-enzymatically to quinolinic acid and enzymatically to picolinic acid. In a subsequent publication, Mehler and May presented evidence which proved that the intermediate, 2-acroleyl-3-aminofumarate, is decarboxylated by the enzyme picolinic carboxylase to form picolinic acid. The results of this experiment also suggested that the formation of picolinic acid by decarboxylation of the intermediate is a major step in the metabolism of 3-hydroxyanthranilic acid.

Following the experiments of Mehler and co-workers, Suhadolnik et al. studied the activity of picolinic carboxylase in liver extracts from ten different species. These investigators discovered that the activity of the enzyme varied markedly among species. The activity was greatest in cat liver and least in mouse and pig liver. However, the authors pointed out that the levels of activity observed did not necessarily reflect the true concentration of the enzyme because they had not treated the liver extracts with reduced glutathione which is required to maximize picolinic carboxylase activity.

The experiments, conducted in vitro, by Mehler and co-workers and Suhadolnik et al. established the fact that mammalian liver contains an enzyme that decarboxylates an intermediate of tryptophan metabolism to form picolinic acid. However, the results of Suhadolnik et al. obtained with intact rats and cats caused them to question the importance of picolinic carboxylase activity. These investigators injected either tritium-labeled 3-
hydroxynanthranilate or $^{14}$C-labeled tryptophan into cats and rats and then collected urine for analysis of picolinic acid. When the labeled 3-hydroxynanthranilate was injected into the animals, approximately 4% of the dose was recovered as picolinic acid in the urine from both species. When the labeled tryptophan was injected, no labeled picolinic acid was recovered in the urine from either species. Because a 30 mg test dose of intraperitoneally administered picolinic acid was recovered nearly quantitatively from the urine, Suhadolnik et al. concluded that picolinic acid is not a significant end product of tryptophan in the rat or cat. The authors did not, however, discuss the possibility that the poor recovery of labeled picolinic acid in their experiments with intact animals could have resulted from feed back regulation, hormonal control of picolinic carboxylase or the inability to accurately quantitate circulating picolinic acid levels from urinary collection.

McDaniel et al. discovered that rats made diabetic with alloxan excreted less N-methyl nicotinamide than normal rats. This observation prompted Mehler et al. to examine the effect of the diabetic state on the activity of picolinic carboxylase. Rats were first injected with alloxan after which the animals were killed at varying time intervals and the liver was removed for assay. The activity of picolinic carboxylase in liver extracts from rats made diabetic with alloxan was not altered significantly during the first six days. Thereafter the activity of picolinic carboxylase increased markedly and remained elevated. Administration of insulin to the diabetic rats resulted in a decrease in activity but the level remained slightly higher than that in normal rats. These observations proved that insulin, either directly or indirectly, regulates the activity of picolinic carboxylase.

In a later study, Mehler et al. discovered that adrenal steroids are somehow involved in the regulation of picolinic carboxylase activity. When diabetic rats were adrenalectomized the marked elevation in enzyme activity did not occur. When the diabetic adrenalectomized rats were treated with cortisone, the activity of the enzyme increased to the levels observed in diabetic rats. However, picolinic carboxylase activity was not affected when normal rats were treated with cortisone. These observations demonstrate that adrenal steroids either directly or indirectly regulate the activity of picolinic carboxylase while insulin counteracts this effect by decreasing the activity of the enzyme.

A few years after the initial reports of Mehler and Suhadolnik et al. a group of investigators at Kyoto University in Japan described the results they had obtained with picolinic carboxylase. This group substantiated the observations of Suhadolnik et al. by demonstrating that the activity of picolinic carboxylase in liver extracts varied markedly among species. The Japanese group also demonstrated that the activity of the enzyme in rat kidney was nearly ten times greater than the activity in rat liver. They showed that
while the activity of the enzyme increases in livers from diabetic rats, the activity of the enzyme in the kidney from diabetic rats was not significantly altered.

In another report, the Japanese investigators described the characteristics of purified picolinic carboxylase. The enzyme was inhibited by potassium cyanide, p-chloromercuribenzoate, iodoacetate and $\alpha$-hydroxymuconic $\varepsilon$-semialdehyde. The activity of the enzyme was not affected by EDTA or by several other metal-chelating agents. These investigators suggested that picolinic carboxylase catalyzes the conversion of $\alpha$-amino $\beta$-carboxymuconic $\varepsilon$-semialdehyde to another intermediate, $\alpha$-aminomuconic $\varepsilon$-semialdehyde. This intermediate either undergoes non-enzymatic cyclization to form picolinic acid or is attached by a NAD-linked aldehyde dehydrogenase to form alpha-aminomuconic acid which is eventually converted to glutaryl-CoA. These observations demonstrated that the formation of picolinic acid in vivo is regulated by the activity of three separate enzymes; 3-hydroxyanthranilic acid oxygenase, picolinic carboxylase and $\alpha$-hydroxymuconic $\varepsilon$-semialdehyde dehydrogenase. The oxygenase catalyzes the conversion of 3-hydroxyanthranilic acid to an intermediate which spontaneously converts to quinolinic acid unless attacked by picolinic carboxylase. Thus, the quantity of picolinic carboxylase determines how much tryptophan metabolite is directed toward the synthesis of niacin and how much is directed toward the production of glutaric acid. The quantity of the dehydrogenase in turn determines how much intermediate is converted to picolinic acid and how much is further metabolized to glutaric acid. These pathways are illustrated in Figure 1.

As described above, the activity of picolinic carboxylase is known to be regulated by insulin and adrenal steroids. Whether or not any of the metabolites of tryptophan regulate the activity of this enzyme remains to be determined. Recently, Satyanarayana and Narasinga Rao examined the effect of dietary protein and tryptophan on the activity of various enzymes involved in tryptophan metabolism and found that neither protein nor tryptophan affected the activity of picolinic carboxylase in the rat.

ASSOCIATION OF PICOLINIC ACID WITH ZINC ABSORPTION

While picolinic acid has been detected in mammalian organs and the enzyme involved in its synthesis is known to be under hormonal control, the function, if any, of this tryptophan metabolite remains a mystery. Our group began considering picolinic acid as an endogenous metal-chelating agent after we detected the pyridine carboxylic acid in human milk. For several years we had tried unsuccessfully to identify a low-molecular weight zinc-binding ligand that had been detected in rat intestine and dog pancreas. We began
FIGURE 1 Metabolism of tryptophan to picolinic acid.

Concentrating our efforts on the identification of this elusive ligand in human milk after malabsorption of zinc was associated with the genetic disease known as acrodermatitis enteropathica. Acrodermatitis enteropathica (AE) is an autosomal recessive disease characterized by diarrhea, skin rash, poor growth, hair loss and susceptibility to infection. In 1973, Moynahan and Barnes reported that the symptoms of AE could be ameliorated with oral
zinc supplementation. This observation was later substantiated by several clinical trials. In 1975, Lombeck et al. demonstrated that symptoms of AE result from impaired zinc absorption. Prior to the report of Moynahan and Barnes, AE had been successfully treated with either human milk or diiodohydroxyquinoline, a potential metal-chelating agent. Moreover, the symptoms of AE do not generally appear until after the child is weaned. These observations suggested that human milk contains a chelating agent that facilitates zinc absorption because the concentration of zinc in human milk does not exceed that of cow's milk.

In our attempt to isolate and identify zinc-binding ligands in human milk we adopted a rather unconventional, but effective method. The technique we used was based on the concepts of a method first described by Hummel and Dryer in which the gel filtration medium was saturated with buffer before and during chromatography. In our adaptation of the technique, gel filtration medium was saturated with zinc ion to prevent separation and/or loss of zinc and ligand during chromatography. After each step used for purification (ultrafiltration, cation exchange and anion exchange chromatography), the fractions collected were chromatographed through a gel saturated with zinc. By use of this technique, we were able to isolate a zinc-binding ligand in purified form that could be subjected to analyses of composition.

The clinical observations of Robertson et al. greatly simplified the task of identifying the zinc-binding ligand we had isolated from human milk. These investigators found evidence for impaired tryptophan metabolism in children who were affected with AE and suggested that the genetic defect in this disease involved an enzyme in the pathway from tryptophan to nicotinic acid. We believed that this malady resulted from an impaired production of an essential zinc-binding ligand. Therefore, we considered the possibility that a tryptophan metabolite might be the elusive zinc-binding ligand. Two metabolites that immediately caught our attention were the pyridine carboxylic acids: picolinic acid and quinolinic acid. When we compared the chemical properties of these two compounds with those of the ligand isolated from human milk we discovered that the zinc-binding ligand was identical to picolinic acid.

Shortly after we had identified picolinic acid in human milk, we began collaborating with Dr. I. Krieger at the Children's Hospital of Michigan and subsequently identified picolinic acid in a pancreatic extract. Krieger had discovered that the symptoms of AE in a young patient could be prevented by either administration of a pancreatic extract known as Viokase (Viobin, Monticello, IL) or oral administration of 60 mg zinc daily as zinc sulfate. Because the daily intake of zinc was only 5 mg per day when the child was given Viokase, Krieger asked us to test this extract for the presence of a zinc-binding ligand. We identified picolinic acid in Viokase and found that the concentration was 3.4 µmoles per gram. Since both human milk and the
pancreatic extract contained picolinic acid and both had been used effectively to treat the symptoms of AE, we began to suspect that picolinic acid may play a role in the absorption and transport of zinc and perhaps other trace metals.

METAL-BINDING PROPERTIES OF PICOLINIC ACID

The chemical properties of picolinic acid make this compound an ideal metal-binding ligand. The fact that picolinic forms stable metal chelates was first reported over 100 years ago.\(^2\) Since that time several studies have been conducted to determine the pK values of picolinic acid and the stability constants of several metal chelates.\(^2\) Values ranging from 1.01 to 1.6 have been reported for the pK of the carboxylic acid group and values of 5.23 to 5.44 have been determined for the pyridine nitrogen. Stability constants determined with biologically essential metals reveal the following order: Cu\(^{2+}\) > Fe\(^{3+}\) > Fe\(^{2+}\) > Zn\(^{2+}\) > Mn, Mg, Ca. Stability constants reported for copper chelates range from log \(\beta_2\) = 12.2 to log \(\beta_2\) = 16 while the stability constants determined with zinc chelates range from log \(\beta_2\) = 9.4 to log \(\beta_2\) = 10.01.\(^2\) The experiments of Paul \textit{et al.}\(^2\) suggest that picolinic acid acts as a bidentate ligand in which the metal complex is formed by coordination between the pyridine nitrogen and the carbonyl oxygen on the adjacent carboxylic acid group (Figure 2). Thus, at physiological pH, picolinic acid is fully dissociated and forms very stable complexes with biologically essential metals such as copper, iron and zinc.

EFFECTS OF EXOGENOUS PICOLINIC ACID

To determine whether or not picolinic acid would affect trace metal metabolism, we first conducted a series of experiments with rats fed diets that contained supplemental picolinic acid.\(^2\) Rats fed a diet formulated with casein and supplemented with picolinic acid (0.2 mg/g diet) gained significantly more weight than rats fed the same diet without supplemental picolinic acid. The kidney zinc concentration of the supplemented rats was significantly greater than that of the unsupplemented rats. Moreover, the rats fed the diet supplemented with picolinic acid absorbed significantly more dietary zinc than the rats fed the unsupplemented diet. In the same experiment, rats fed a diet formulated with soy-protein and supplemented with picolinic acid gained significantly more weight than rats fed the same diet without added picolinic acid. The kidney zinc concentration of the supplemented rats was significantly greater than that of the unsupplemented rats.

In another experiment, the zinc concentration of liver and kidneys was
determined in rat pups suckling dams given 10 µg Zn/ml in drinking water as either zinc dipicolinate or zinc acetate during the last week of gestation and for 5 days of lactation. The zinc concentrations of both liver and kidneys from the 5-day-old pups suckling dams fed the zinc dipicolinate solution were significantly greater than the zinc concentration of these tissues from pups suckling dams given a solution of zinc acetate.27

Several factors are known to inhibit absorption of dietary zinc and included in these components is dietary iron.28-30 Recently we discovered that either picolinic acid or vitamin B-6 would overcome the competition between dietary iron and zinc.31 Daily zinc absorption in rats fed a diet that contained 30 ppm Fe and 4 ppm pyridoxine-HCl was 242 µg. When rats were fed a high-iron diet (220 ppm Fe) that contained 4 ppm pyridoxine-HCl, daily zinc absorption was only 165 µg. However, when the pyridoxine-HCl content was increased to 10 ppm and 40 ppm, daily zinc absorption was 197 µg and 223 µg respectively. Furthermore, daily zinc absorption was increased to 280 µg when 200 ppm picolinic acid was added to the high-iron diet that contained 4 ppm vitamin B-6.

Several years ago, Hsu32 discovered that the zinc concentration of several organs from rats fed a pyridoxine-deficient diet was significantly less than that of pair-fed control rats fed a pyridoxine-supplemented diet. Diets deficient in either thiamin or riboflavin did not produce the same effect. Recently, we observed that growth rate and kidney zinc concentration are significantly greater in rats fed a vitamin B-6 deficient diet supplemented with picolinic acid than in rats fed the same diet without supplemental picolinic acid.26 Moreover, the retention of zinc in rats is directly related to the level of dietary vitamin B-6.31 When rats were fed a diet that contained only 2 ppm vitamin B-6, the half-life of orally administered 65Zn was 37.62 days. When picolinic acid (200 ppm) was added to this same diet, the half-life of 65Zn was increased to
90.83 days and when the level of vitamin B-6 was increased to 10 ppm and 40 ppm the half-lives were 59.08 days and 86.33 days respectively.

The observations described in the preceding paragraphs demonstrate that zinc absorption and retention are directly related to the level of dietary vitamin B-6. Vitamin B-6 is known to be a cofactor for at least one of the enzymes (kynureninase) involved in the conversion of tryptophan to picolinic acid. Furthermore, supplemental picolinic acid ameliorates the effects on zinc metabolism observed when rats are fed diets that contain deficient or marginal levels of vitamin B-6. Thus, the effect of vitamin B-6 on zinc absorption and retention is probably related directly to the synthesis of endogenous picolinic acid.

Experimental results obtained with rats fed low-protein diets provides further evidence that zinc absorption is influenced by endogenous picolinic acid. Van Campen and House\textsuperscript{33} first demonstrated that rats fed a diet containing only 5% protein absorbed significantly less zinc than rats fed a diet that contained 15% protein. We found that rats fed a 5% casein diet absorbed only 59% of the daily zinc intake. When the 5% protein diet was supplemented with enough tryptophan to equal that in a 20% casein diet the rats absorbed 98% of the daily zinc intake. Furthermore, rats fed the 5% casein diet supplemented with 200 ppm picolinic acid absorbed 87% of the daily zinc intake.\textsuperscript{34} Since tryptophan is the precursor of picolinic acid in vivo, these observations support the hypothesis that endogenous picolinic acid is involved in zinc metabolism.

**PICOLINIC ACID AND ZINC METABOLISM IN HUMANS**

Our experiments with picolinic acid have not been confined to animals. Shortly after we detected picolinic acid in human milk we began collaborating with Drs. Ingeborg Krieger and Ralph Cash of the Children's Hospital of Michigan in Detroit in an attempt to determine the relationship between zinc and picolinic acid in humans.\textsuperscript{35} Three children known to have acrodermatitis enteropathica were treated with oral daily doses of zinc dipicolinate. One patient remained symptom free on a dose of 10 mg zinc per day in the form of zinc dipicolinate. Prior to this study, the patient had required at least 30 mg zinc per day in the form of zinc sulfate. A second patient required 45 mg zinc per day in the form of sulfate to arrest symptoms but when treated with zinc dipicolinate required only 15 mg zinc per day. The third patient required 15 mg zinc per day when being treated with zinc sulfate but the requirement was reduced to only 5 mg zinc per day when zinc dipicolinate was used as therapy.
During the course of the studies described above, the picolinic acid concentration of the plasma in the three patients and in twelve control children was assayed by high pressure liquid chromatograph (hplc). The mean concentration of picolinic acid in the plasma from symptom-free children with AE who were being treated with oral zinc sulfate (30–45 mg Zn/day) was $4.8 \pm 4.3$ (SD) $\mu$moles/liter. The picolinic acid concentration in plasma from the control children was $12.4 \pm 3.3$ (SD) $\mu$moles/liter. In addition, kynurenine was not detectable in the plasma of any of the 12 control subjects but the plasma from children with AE contained $20.0 \pm 5.2$ (SD) $\mu$moles/liter.

The results described above demonstrate that oral zinc is utilized in humans more efficiently when complexed with picolinic acid. Moreover, the results support the hypothesis that tryptophan metabolism is impaired in children affected with acrodermatitis enteropathica. Our results and those of Robertson et al. suggest that the primary lesion in AE patients is a defective enzyme in the tryptophan metabolic pathway. This defect results in a decreased production of picolinic acid which facilitates zinc absorption, reabsorption, transport and retention in cells.

The effectiveness of zinc dipicolinate as an oral zinc supplement has been observed in patients with pancreatic insufficiency. Boosalis et al. compared zinc absorption among healthy control subjects and patients with alcoholic cirrhosis or pancreatic insufficiency by use of a zinc tolerance test. Twenty-five mg zinc as either zinc sulfate or zinc dipicolinate was administered orally two days apart to fasted subjects after which blood was collected over a period of 4 hr. Peak zinc levels in healthy controls were observed after 2 hr and there was no significant difference between absorption of zinc sulfate and zinc dipicolinate. Zinc absorption was significantly decreased in alcoholic cirrhotics and there was no significant difference between absorption of zinc sulfate and zinc picolinate. Absorption of zinc sulfate was significantly decreased in patients with pancreatic insufficiency. However, absorption of zinc dipicolinate in these patients was normal. These results demonstrate that patients with pancreatic insufficiency have an impaired capacity to absorb zinc but zinc dipicolinate reverses this absorptive defect.

Experiments conducted at our laboratory demonstrate that oral picolinic acid increases the retention of endogenous zinc in adult men. Seven adult males were fed meals that contained corn cereal intrinsically labeled with $^{65}$Zn. Thereafter, retention of $^{65}$Zn was monitored weekly by whole body scintillation counting. Sixty-days after ingestion of the $^{65}$Zn, the subjects were given 3.3 mg picolinic acid three times daily with meals for three to six weeks. In six of the subjects, retention of $^{65}$Zn was increased over a three week period. Prior to daily oral ingestion of 10 mg picolinic acid the half-lives of $^{65}$Zn ranged from 150–491 days. During three weeks of picolinic acid supplementation the half-lives were 835 days or greater. However, after three
weeks of picolinic acid supplementation the half-lives began to approach the pre-supplementation values in most of the subjects.

SUMMARY

Experiments with both animals and humans suggest that endogenously produced picolinic acid may function as a chelating ligand in the metabolism of zinc and perhaps other essential metal ions. Zinc absorption and retention are depressed in animals fed diets that contain suboptimal levels of tryptophan, the precursor of picolinic acid. Zinc absorption and retention are also decreased in animals fed diets that contain marginal or deficient levels of vitamin B-6, a cofactor of at least one of the enzymes in the pathway from tryptophan to picolinic acid. Zinc absorption and retention can be restored to near normal in rats fed diets that contain marginal or deficient levels of either tryptophan or vitamin B-6 by the addition of picolinic acid to the diet.

The concentration of picolinic acid is decreased in the plasma from children affected with acrodermatitis enteropathica, an inborn error of metabolism which results in impaired zinc absorption. Furthermore zinc dipicolinate is more efficacious than zinc sulfate in the treatment of this disease. Absorption of zinc sulfate is decreased in patients with pancreatic insufficiency but absorption of zinc dipicolinate is normal in these patients. Oral picolinic acid supplementation in healthy adult males resulted in an increase in zinc retention.

At physiological pH, picolinic acid is fully dissociated and forms stable complexes with essential metals. Picolinic carboxylase, the enzyme involved in the conversion of tryptophan metabolites to picolinic acid, is regulated by insulin and corticosteroids. Marked changes in metal homeostasis have been detected in diabetes and adrenal insufficiency. Whether or not these changes are related to alterations in picolinic acid synthesis are not yet known. Future studies with both experimental animals and humans will probably prove that picolinic acid has a key role in the absorption and reabsorption of specific metal ions as well as the retention of these metal ions in the cells of the body.

References