Pathobiochemistry of vitamin B12

Background information

Absorption of vitamin B12. Vitamin B12 is readily absorbed when administered subcutaneously or intramuscularly. Absorption from the gastrointestinal tract is more complex. A glycoprotein secreted by the gastric mucosa, called intrinsic factor, binds B12 and enhances its absorption by the ileum. Insufficient amounts of B12 are transported by other mechanisms. In the absence of intrinsic factor, B12 cannot be absorbed unless it is given in amounts greater than those present in the ordinary diet.

Structure and functions of vitamin B12 coenzymes. Most of the modern literature now designates the group of cobalt-containing compounds to which vitamin B12 belongs as cobalamin. The structure of a cobalamin is given in textbooks, but it is convenient to abbreviate it as follows:
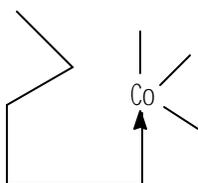
The four straight lines on the right and left sides, which are directed at the cobalt atom, represent bonds to four nitrogen atoms in the corrin ring structure. The corrin ring system has structural features in common with the porphyrin ring system but also differs in many respects. The arrow at the bottom of the cobalt atom represents a coordinate covalent bond from a nitrogen atom of the dimethylbenzimidazole moiety. A corrinoid compound that contains dimethylbenzimidazole is called a cobalamin. Vitamin B_{12} as it occurs in vitamin capsules, is cyanocobalamin and is designated as:
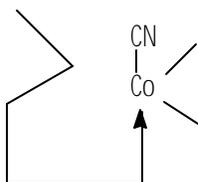
The cyanide substituent arises during the isolation of the vitamin from its natural commercial sources, usually bacteria. It is not found on cobalamin that occurs in the body and is not toxic in the usual concentrations of cobalamin taken in vitamin capsules. The three cobalamins found in the body are designated as follows: (see next page)

Aquocobalamin
5'-Deoxy-5'adenosylcobalamin
(Adenosylcobalamin)
Methylcobalamin

Methyl-trap hypothesis

5-Methyl THF is involved in the synthesis of methionine by the liver. It is formed and utilized as follows:

It should be recalled that although methionine is an essential amino acid, only its homocysteine portion cannot be synthesized by humans.

The enzyme 5-methyl THF methyltransferase has a requirement for methyl B12 coenzyme. This enzyme catalyzes the methylation of homocysteine shown in the above reaction, which is the only

significant metabolic reaction in humans that uses both folate and B12 coenzymes. Consequently, a vitamin B12 deficiency may result in the conversion of all THF to 5-methyl THF. The 5-methyl THF methyltransferase reaction is the major and perhaps the only, metabolic reaction that can use 5-methyl THF. If this reaction is blocked or slowed down as a result of a deficiency of vitamin B12, 5-methyl THF accumulates and the THF needed for other folate-requiring reactions is not formed. Eventually, most of the folate in the body ends up as 5-methyl THF. Even in normal individuals, the major blood and tissue form of folate is 5-methyl THF; thus any impairment of its use can lower greatly the concentration and availability of other folate coenzymes.

This hypothesis, the methyl trap hypothesis has been offered to explain why certain anemic conditions respond to either vitamin B12 or folic acid and particularly to a combination of the two. For example , the administration of folate will alleviate the hematologic symptoms of the pernicious anaemia caused by vitamin B12 deficiency but does not prevent the irreversible neurologic damage caused by a continued lack of vitamin B12.

Case 1 Defect in B12 coenzyme synthesis
A male infant hospitalized from age 4 wk until his death at age 7« wk found to have very low serum, brain, and liver concentrations of methionine. He had methylmalonic aciduria. Serum homocystine and cystathionine were elevated, but other amino acid concentrations were normal. The total B12 content of the liver and kidneys was normal, but the concentrations of 5'-deoxyadenosylcobalamin were less than 10% of controls. Folic acid was administered on the fifteenth day of hospitalization; 5 days later the serum 5-methyl THF was well above normal. Postmortem examination of extracts from the liver and kidneys were abnormally low in 5-methyl THF methyltransferase.
Case description adapted from Mudd HS. et al.: Biochem. Biophys. Res. Comm. 1969, 35: 121,

Biochemical questions

1. What metabolic abnormality is suggested by the low methionine and high homocystine levels? What is the coenzyme?

2. What are two well-established enzymatic reactions in animals that require B12-containing coenzymes?

3. Considering the laboratory values presented, what reaction would you expect to be blocked in this infant? Explain.

4. Are these findings consistent with the methyl-trap hypothesis? Explain.

5. Would you try to treat a patient suffering in the same disease with carnitine? Why would you consider the carnitine administration?

References

Rosenblatt DS and Cooper BA: Inherited disorders of vitamin B12 metabolism, Blood Revs 1:172-182, 1987.

Rosenberg L., Disorders of propionate and methylmalonate metabolism In Stanbury et al, editors: The metabolic basis of inherited disease, ed 5. New York, 1983, 474-497. McGraw-Hill Book Co.

Roe C.R. et al. Metabolic response to carnitine in methylmalonic aciduria. Arch. Dis. Child. 1983, 58. 916-920.

Case 2   Methylmalonic acidemia

Earlier this female patient had a child who died at age 3 mo of severe acidosis and dehydration. A diagnosis of methylmalonic acidemia was made posthumously. The patient was now pregnant and concerned that the fetus might have the same disease. At l9 wk gestation, amniotic fluid cells were assayed for propionate and succinate oxidation, vitamin B12 (cobalamin) coenzyme biosynthesis, and methylmalonyl CoA mutase activity. Propionate oxidation was much lower than controls, but succinate oxidation was normal. Radioactivity from 57Co-labeled cobalamin was found in methyl cobalamin but not in deoxyadenosyl cobalamin; however, methylmalonyl CoA mutase activity was normal in cell lysates supplemented in vitro with substrates and cofactors. This enzyme requires adenosyl cobalamin as a coenzyme. The patient's urine contained large amounts of methylmalonic acid, which it did not before pregnancy.

Biochemical questions

l . What amino acids produce propionyl CoA in humans? What would happen if a person ate a diet lacking these amino acids?

2. How can you explain the high concentration of methylmalonic acid in the urine when amniotic cells showed normal activity of methylmalonyl CoA mutase? Assume that all metabolic defects are present in the fetus and not in the mother.

3. Why was one cobalamin coenzyme labeled after administration of radioactive vitamin B12 and not the other coenzyme?

4. At 32 wk gestation the mother was excreting approximately six times the normal amount of methylmalonate in her urine. She was given oral cyanocobalamin, 10 mg/day. One week after therapy was started, the methylmalonate concentration dropped to threefold above normal and by delivery at 41 wk was almost normal. The infant was born in excellent condition; however, cultured skin fibroblasts from the infant displayed the same abnormalities as the amniotic cells. The infant

was maintained on a low-protein diet (1.5 g/kg/day) but was not treated further with cobalamin. Why was the low-protein diet used, considering that the infant would be growing rapidly?

5. During the next 6 wk the infant cleared the vitamin B12 absorbed from the mother before birth. At the same time the protein in the diet was increased to 2.5 g/kg/ day, and cobalamin was administered intramuscularly for 11 days. Considering the enzymes involved, explain how high levels of vitamin B12 result in decreased excretion of methylmalonate.

6. At 19 mo of age the child was in the 75th percentile for weight, length, and head circumference, and development was normal. She was being maintained on a lowprotein diet, and continuous cobalamin therapy had not been necessary. Do you think that this child was homozygous or heterozygous for methylmalonic acidemia? Explain.

7. If, as an adult, this girl became obese and desired to lose weight, how and for what reasons would you treat her differently from other obese patients?

References

Rosenberg L., Disorders of propionate and methylmalonate metabolism In Stanbury et al, editors: The metabolic basis of inherited disease, ed 5. New York, 1983, 474-497. McGraw-Hill Book Co.

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Case discussion
Defect in B12 coenzyme synthesis
1. Low methionine and high homocystine levels In this patient these low levels suggest a defect related in some way to the synthesis of methionine from homocysteine. Homocystine, the disulfide form of homocysteine, often is present in situations where homocysteine accumulates, because the sulfhydryl group of the latter compound is very sensitive to oxidation. Because the vitamin B12 findings are also abnormal, it is possible that the B12 coenzyme-directed synthesis of methionine is disturbed. This prediction was borne out by a decreased activity of 5-methyl THF methyltransferase. The genetic defect, however, could be in either the enzyme itself or the availability of a coenzyme essential to the reaction.
2. Coenzyme reactions Only methylcobalamin and adenosylcobalamin are coenzymes. These coenzymes are normally synthesized from dietary cyanocobalamin or aquocobalamin. Two enzymatic reactions have been conclusively shown to require cobalamin coenzymes in humans. One requires adenosylcobalamin, and the other methylcobalamin.
Adenosylcobalamin is required for the isomerization of methylmalonyl CoA to succinyl CoA. This reaction is important in using the methylmalonyl CoA synthesized by the carboxylation of propionyl CoA so that odd chain fatty acids and certain amino acids, whose catabolism yields propionate, can be metabolized. The methylmalonic aciduria seen in this case is suggestive of a defect in this 5'-deoxyadenosylcobalamin requiring reaction. The reaction catalyzed by 5-methyl THF-homocysteine methyltransferase contains methylcobalamin as a tightly bound prosthetic group. When isolated, the holoenzyme appears to have the cobalamin present as aquocobalamin. To activate the prosthetic group, it must be reduced with a reduced flavin and methylated with 5-adenosylmethionine (S-AdoMet).
Aquocobalamin + 5-AdoMet+FADH2-->Methylcobalamin + S-Adenosylhomocysteine+FAD

The methylcobalamin prosthetic group can then transfer its methyl group to homocysteine and in turn be remethylated by 5-methyl THF:
1. Methylcobalamin + Homocysteine-->Methionine + Reduced cobalamin (cob[I]alamin)
2. Cob[I]alamin + 5-methyl THF -->Methylcobalamin + THF
Although the transferase can catalyze many cycles of methyl transfer in this way, the prosthetic group occasionally reverts to an inactive form, again necessitating further reactivation by FADH2 and 5-AdoMet. The chemical details of this inactivation-reactivation process are obscure, but it is probably related to the very marked nucleophilic properties of the cob[l]alamin intermediate that occasionally cause the latter to undergo a side reaction.
3. The blocked reaction Considering these facts, the data in this case indicate a defect in the conversion of cobalamin to both its coenzymatic forms. Total cobalamin content of liver and kidneys was normal, but 5 -deoxyadenosylcobalamin was low; thus the methylmalonic aciduria resulted. Although methylcobalamin was not determined in this case, a similar more recent case showed it to be low. Thus the low activity of the 5-methyl THF methyltransferase was probably caused by the lack of methylcobalamin. The reactions involved in the conversion of aquocobalamin to cobalamin coenzymes are not well understood in the human. Probably the defect in this case is in a reaction that produces an intermediate common to the synthesis of both coenzymes. This

intermediate (see previous numbered equation) is probably a reduced cobalamin, and the reducing agent is likely FADH2

4. The methyl-trap hypothesis Even though the lack of activity of 5-methyl THF-homocysteine methyltransferase is secondary to a primary defect in the synthesis of cobalamin methyl, these results are in accord with the methyl-trap hypothesis. Thus liver methionine was low and both homocystine and 5-methyl THF concentrations were high because the methylcobalamide-dependent transferase reaction was blocked. lt is expected that 5-methyl THF accumulated, and the concentration of other folate derivatives decreased, because all the folate was "trapped" as methyl THF. If the patient had lived, it is expected that the deficiency of folate derivatives other than 5-methyl THF would have produced megaloblastic anemia.