Repression of the Overproduction of Porphyrin Precursors in Acute Intermittent Porphyrin by Intravenous Infusions of Hematin

(δ-aminolevulinic acid/porphobilinogen/heme/cerebrospinal fluid)

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ABSTRACT In a patient with a severe attack of acute intermittent porphyria, hematin given intravenously caused marked diminution of serum δ-aminolevulinic acid and porphobilinogen. The decline of aminolevulinate was more rapid than that of porphobilinogen. After 2 days of hematin administration, about 5 days were required for δ-aminolevulinic acid, and 11 days for porphobilinogen to return to the concentrations that were detected before treatment. Urinary excretion of both compounds also decreased after hematin administration. Considerable amounts of porphobilinogen were also found in the cerebrospinal fluid of the patient.

Acute intermittent porphyria is characterized biochemically by the increased urinary excretion of the porphyrin precursors δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). This is believed due to a marked, genetically mediated increase of hepatic ALA synthetase (1-4), which is normally the first and rate-controlling enzyme of heme biosynthesis. A decreased activity in the hepatic conversion of PBG to porphyrins in a patient with acute intermittent porphyria has recently been noted (5-7). This suggests a partial block in heme biosynthesis in this case that may relate to the observed induction of hepatic ALA synthetase, the end-product heme having been shown to repress the synthetase production both in vitro (8-10) and in vivo (11, 12). These considerations induced us to study the effect of hematin on the induction of the synthetase in a patient experiencing a devastating attack of acute intermittent porphyria, after numerous other modes of therapy had failed.

MATERIALS AND METHODS

Case Protocol. M. M., 33, a white woman of Irish extraction, was hospitalized at the NIH Clinical Center, from February 26 to May 29, 1971. The diagnosis of acute intermittent porphyria was first made in 1955 when she developed abdominal pain and passed dark urine, which gave a strongly positive Watson-Schwartz test for PBG. Her family history was positive and the diagnosis was confirmed repeatedly by demonstration of the characteristic biochemical abnormalities in her urine (13).

Subsequent attacks, often with onset shortly before the menses, were characterized by abdominal pain, nausea, constipation, occasional vomiting, and when severe, in addition by generalized pain, tenderness, dysesthesias, and weakness. Also, mild hypertension had been noted during an attack in 1963. In 1966, the patient suffered a severe paralytic attack with gradual recovery.

Her final attack began in early February, 1971. Shortly before admission, her blood pressure was 290/160. Admitted to the Clinical Center on February 22, 1971, she was very ill, apprehensive, and perspiring; blood pressure was 256/150, pulse was 114. The optic fundi showed severe hypertensive changes with marked arteriolar narrowing, hemorrhages, and exudates.

Neurological examination revealed generalized tenderness, a decrease in vibratory sense in both feet, and mild to moderate weakness in all extremities, worse distally than proximally. She was unable to extend the hands or wrists and had slight weakness of ankle and foot movements, but other motor functions were good.

Urinalysis showed a large amount of PBG and 1+ protein; the blood-urea nitrogen was 47 mg/100 ml; serum creatinine was 2.2 mg/100 ml. 24-hr endogenous creatinine clearances during the first 2 weeks of hospitalization were 18 and 28 ml/min.

Various forms of therapy met with no success. These included reserpine, guanethidine, and mecamylamine for the hypertension; chlorpromazine and meperidine for the porphyric distress; a high carbohydrate intake (up to 450-600 g/day) with and without insulin, with intention of repressing the induction of ALA synthetase by means of the "glucose effect" (13); the addition of glycerol with the same intent; testosterone propionate; vitamin E; copper sulfate; and adenosine monophosphate. The clinical condition of the patient deteriorated slowly, but inexorably. Her weakness progressed steadily from the distal to proximal muscle groups of the extremities; she became bedridden and totally incapable of self-care.

By April there was dysphagia and inability to clear bronchopulmonary secretions. Tracheostomy and continuous assisted ventilation were required. The pulse remained fast, the patient perspiring and extremely apprehensive.

Because of this deterioration despite all treatment, intravenous administration of hematin was begun on April 13, with the informed consent of the patient and her family. Initially, 250-mg amounts were given twice daily on April 13 and 15. Inadvertently, on April 14 two doses were given,
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FIG. 1. Concentrations of ALA and PBG in the serum and cerebrospinal fluid of the patient. The solid line represents PBG, and the dashed line represents ALA. The circles are serum values; the squares are values of cerebrospinal fluid in µg/100 ml. The numbers above the graph refer to the dosage of intravenous hematin in mg. The rectangle with hatched lines represents dialysis. The first part of each serum curve is dashed to emphasize that only one sample was assayed before administration of the infusions. The curve for ALA is dashed from 5/20 to 5/21 to indicate that a serum sample was not analyzed for ALA just before the hematin was infused on 5/21.

in part subcutaneously. There was no apparent clinical response. The biochemical response is discussed below.

On April 16, the patient developed acute gastric bleeding due to multiple "stress" ulcers. This required sub-total gastrectomy followed by severe renal failure and complicated by gram-negative bacterial sepsis and disseminated intravascular coagulation. After appropriate treatment, the latter conditions improved, but the renal failure progressed and peritoneal dialysis was performed on April 30–May 2. This corrected volume overload and congestive heart failure with lowering of the blood urea-nitrogen (250–63 mg/100 ml) and serum creatinine (6.4–3.0 mg/100 ml), but did not improve the neurological dysfunction. Immediately after dialysis, renal function remained very poor (endogenous creatinine clearance of 1–5 ml/min) with rapid reaccumulation of urea and creatinine in the blood. The patient became totally anuric on May 19 and remained so until death.

On May 6 she became comatose and developed opisthotonus. Hematin was resumed on May 7, again without clinical response, and the patient died on May 29, of profound central nervous system failure attributed to acute porphyria and uremia. At autopsy, the kidneys showed evidence of essential hypertension and marked tubular injury.

Chemical Procedures. Urines were collected, refrigerated, and assayed for porphyrin precursors within 3 days of collection (14). The dialysate was collected in clear glass bottles and refrigerated (4°C) for 2 weeks before analysis. Blood-serum samples were frozen and kept in the dark until assayed. ALA and PBG of blood serum and cerebrospinal fluid were determined by the method of Miyagi et al. (7), and of dialysate by the method of Marver et al. (14).

Three samples of cerebrospinal fluid were assayed for PBG and ALA. In one, the minute quantity available sufficed only for a qualitative Watson-Schwartz test (13) for PBG. The others were subjected to the quantitative procedure (7).

Hematin was prepared as follows: Hemin was crystallized (15) from packed human erythrocytes from blood that was Australia-antigen negative. It was recrystallized (16), and an appropriate weight was dissolved in 1% Na₂CO₃ with brief warming to 40–60°C (final concentration: 10 mg/ml). The pH was adjusted to 7.5–8.0 with 10% HCl, and the solution was then passed through a 0.20-µm Millipore filter and ultra-purified transferred to a sterile brown vial. The solutions were nonpyrogenic and bacteriologically sterile. Each dose of hematin was freshly prepared just before use and was given over a 1–2 hr period, in amounts that earlier experience had shown to be innocuous in normal subjects (17).

RESULTS

The initial infusion of 250 mg of hematin over 2 hr on April 13 produced a rapid decrease of urinary excretion of ALA and PBG. During the prior 45 days, the rate of urinary ALA excretion ranged from 286 to 2660 µg/hr and PBG from 2000 to 9520 µg/hr (measured in urine samples collected in 2- to 24-hr aliquots). After the infusion on April 13, these values decreased to nadirs of 55 µg/hr for ALA and 630 µg/hr for PBG, in other words, less than 1/4 and 1/2, respectively, of the lowest values previously observed. There were no effects of this nor later hematin infusions on pulse rate, blood pressure, or urinary flow rates.

The second dose of hematin again caused a decrease in the rate of excretion of ALA, but that of PBG rose in a manner roughly reciprocal to the fall of ALA, probably due to conversion of ALA to PBG.

On May 15, 250 mg of hematin was infused twice. After the first, there was again a rapid, though brief, decline in the rates of excretion of ALA and PBG. Shortly after onset of the second infusion, the excretion rate of ALA at once declined sharply to 100 µg/hr, whereas the rate for PBG again rose briefly during the hour after the infusion, but it had fluctuated irregularly before and after this infusion.

Because of the profound and progressive deterioration of renal function, later urinary values were less decisive. Nevertheless, until the onset of complete anuria, an effect of hematin on urinary excretion of ALA and PBG was still demonstrable, even in the presence of advanced renal failure. Before the administration of hematin on May 8 and 9, the base-line rates were 10–30 µg/hr for ALA and 250–500 µg/hr for PBG. After the administration of hematin on these dates, the rates declined to 5 µg/hr for ALA, and 50 µg/hr for PBG.

The difficulty in interpreting the urinary excretion data, due to progressive deterioration of renal function, induced us to study the longer-term effects of hematin on the serum concentrations of ALA and PBG (Fig. 1). Although the number of samples obtained during the early portion of the study was small, the data in Fig. 1 indicate that the hematin infused on April 13–15 caused a decline in serum concentrations of both ALA and PBG.

The effects of peritoneal dialysis on serum ALA and PBG were negligible (Fig. 1). The clearances of ALA and PBG by dialysis were estimated to be about 4–5 ml/min, (rate of exchange of dialysis fluid of 2.0–2.5 ml/hr). The renal clearance of PBG on April 19 was 5.9 ml/min.

The infusion of 500 mg of hematin on May 7 produced a rapid and profound drop in serum ALA concentration (Fig. 1). The range of values for four sera obtained May 7 just before the infusion was 73–76 µg/100 ml. A nadir of 9 µg/100 ml was reached 8 hr after the hematin administration and the concentration remained close to this for the next 16 hr. A decrease was also observed in serum PBG concentration, but only after a 12-hr period, during which the concentration oscillated, and at the outset actually increased modestly. A similar oscillation of PBG concentration, indeed with an increase of greater
magnitude, was observed in the serum samples obtained on the morning before hematin was given.

On the next day, May 8, 1 g of hematin was infused. The serum ALA concentration increased transiently and the declining PBG curve exhibited a brief plateau (Fig. 1). Then, both continued to decline. On May 9, ALA was 0 (<=1 μg/100 ml), PBG was 375 μg/100 ml. In the next day, the serum ALA rose, while PBG fell to a low concentration of 238 μg/100 ml, 48 hr after hematin was given. Thereafter, it appeared to require about 5 days for ALA and 11 days for PBG to return to pretreatment concentrations (Fig. 1).

The serum ALA and PBG concentrations during the final series of infusions of hematin (Fig. 1; May 21–27), generally show changes similar to those already discussed and the data plotted in Fig. 1 are believed to be sufficiently clear. Worthy of emphasis is that throughout the period from May 20 to 29, the patient was totally without urine output. It is also of interest that just after hematin administration on May 21 and 22, the serum PBG concentration showed minor transient increases interrupting the gradual decrease. It may be noted again that ALA increased, while PBG continued to decrease (May 24–25); there is strong indication that the serum ALA during the period May 23–27 was not responding to the hematin, at least as it had previously. This is discussed later.

The half-times of ALA and PBG in the circulating blood were estimated from values obtained after hematin infusions on May 7–8 and May 21–22 (Fig. 2). At these times, hepatic ALA production was presumably shut off almost completely. While the patient still had some urinary output, the approximate half-times were 3 hr for ALA and 44 hr for PBG (May 7–8); 9 hr for ALA and 51 hr for PBG after the development of complete anuria (May 21–22).

The first sample of cerebrospinal fluid tested for PBG content was obtained on May 7, before the administration of hematin for that day. It was found to give a strongly positive Watson–Schwartz reaction. On May 14, when the serum concentrations of ALA and PBG were increasing (Fig. 1), the ALA and PBG of the cerebrospinal fluid were much lower than in the contemporary serum, being 0 and 79 μg/100 ml, respectively. As seen in Fig. 1, the cerebrospinal fluid concentrations of both precursors on May 26 were much lower than in serum obtained simultaneously.

**DISCUSSION**

This study revealed for the first time that hematin administered intravenously in cases of the human genetic disease, acute intermittent porphyria, suppressed the production of porphyrin precursors, often in marked degree. Discussion of the data is simplified by use of a multicompartmental model for porphyrin precursor metabolism as presented in Fig. 3.

The profound decline of serum ALA and PBG, which was repeatedly demonstrated after the intravenous administration of hematin, is thought to result from repression of hepatic ALA synthetase induction. This is represented in Fig. 3 by a decrease in R1, the rate of ALA synthesis; this leads to a decrease in the liver ALA pool, and thus to a decrease in serum ALA pool. R1, of course, could also decrease due to the inhibition of ALA synthetase by heme. Although hematin in concentrations of 50 μM has been shown to inhibit the partially
purified enzyme (18), no inhibition of hepatic ALA synthetase could be demonstrated in previous studies in rats injected intravenously with hematin, in doses equivalent to those used in this study (11). Hence, in vivo, it appears unlikely that endogenously administered heme in amounts given here would directly inhibit the activity of the intramitochondrial enzyme ALA synthetase. A second cause for a rapid decline of the liver ALA pool when $R_l$ decreases is the conversion of ALA to PBG at a rate of $R_2$, that, though it may be somewhat inhibited by hematin (19, 20), is less so than is $R_l$ (9). In contrast, the hepatic PBG pool will decrease at a slower rate than that of the liver ALA pool, since PBG production from ALA declines primarily as a result of a diminishing liver ALA pool and, also, the metabolic conversion of PBG to porphyrins is known to be impaired in acute intermittent porphyria (5-7). Thus, in this disease, hematin causes the liver ALA pool to decrease more rapidly than the liver PBG pool, because it has a greater influence on suppressing ALA production ($R_l$) than PBG production ($R_2$); PBG concentration is enhanced due also to the inefficient conversion of PBG to porphyrin.

One would expect these differential effects on hepatic pool sizes to be reflected in a more rapid decline of serum ALA than of serum PBG concentrations. As shown in Fig. 2, this is demonstrated by the half-time of ALA in serum, which is about $1/4$ to $1/14$ that of PBG. In addition, however, the serum concentrations represent the difference between the rates of entry of these metabolites into serum and their rates of removal by tissue uptake and urinary excretion; hence, it is necessary to consider these factors as well.

It is clear that the decline of serum ALA and PBG after the administration of hematin is not related to any effect of heme on renal functions: (a) In the early studies, hematin infusions caused diminution of both serum concentrations and urinary excretion rates, while having no effect on urinary flow rate, implying a prerenal mechanism of decrease; (b) In the later studies, the decline occurred in an anuric (essentially nephrectomized) patient. During this time, $k_s$ and $k_b$ (Fig. 3) may be considered to be zero. While the renal component of the net removal rates of serum ALA and PBG can be eliminated, differential rates of removal into nonurinary extravascular compartments could still contribute to differences in serum half-times. Available evidence suggests that the uptake of PBG by tissues (serum PBG pool [$k_t + k_h$]) is less than that of ALA (serum ALA pool [$k_t + k_h$]) (Fig. 3, refs. 21-23). However, the opposite appears to be true in the case of the blood-brain barrier.

Nothing is known about the relative fractional rates of entry of these substances from the tissues into the intravascular compartment [$k_1 + k_h + (k_t + k_h)$]. Thus, when the renal function was nil, and hematin had presumably reduced $R_l$ to a low value, the longer serum half-time of PBG than that of ALA may result from both a higher rate of entry into and a lower rate of removal from the serum of PBG ([liver PBG pool $k_h$ + tissue PBG pool $k_b$]) are greater than [liver ALA pool $k_t$ + tissue ALA pool $k_h$] and [serum PBG pool $k_h$ + $k_t$] is less than [serum ALA pool $k_h$ + $k_t$]).

Interpretation of the smaller oscillations observed in the serum curves of ALA and PBG, is more complex. It has previously been demonstrated that the closed negative-feedback loop, which controls the level of hepatic ALA synthetase, is an oscillatory system; i.e., when the system is perturbed by hematin, oscillations of the level of the enzyme occur (11). It is also known that other factors, including carbohydrate feed-

ing (24-27) and hormones (28-30) affect the operation of the control loop. Whether any of these factors are related to the smaller oscillations observed in Fig. 1 is uncertain. Likewise, the possible effects of hematin on tissue uptake and release of the porphyrin precursors are unknown. Hence, we are unable to explain the smaller oscillations at the present time.

The concentration of ALA and PBG was determined on samples of cerebrospinal fluid and serum obtained simultaneously on two occasions, first while serum concentrations were rising and later while they were falling (Fig. 1). The values for cerebrospinal fluid clearly indicate that PBG can cross the blood-brain barrier, although a considerable gradient exists between serum and spinal fluid. The ratio of cerebrospinal fluid/serum PBG concentrations in both instances was about $1/4$, with the absolute serum concentrations being 363 and 193 mg/100 ml. The ratio for ALA is much lower ("0"/91 and 5/38). In fact the cerebrospinal fluid values for ALA are so low at times when the serum values were considerably elevated as to suggest that only trace amounts cross the blood-brain barrier. Thus, while PBG, as well as ALA, appear not to penetrate certain cells or membranes (21-23), the opposite may be the case for uptake across the blood-brain barrier. It is also barely conceivable that the porphyrin precursors detected in the cerebrospinal fluid may have been synthesized in cells of the nervous system itself.

One of the most striking findings in the present study is that two doses of intravenous hematin (of 500 and 1000 mg) injected on May 7 and 8, respectively, caused the markedly elevated serum ALA concentrations to decline to "0", and also produced a highly significant decrease of serum PBG. Furthermore, serum concentrations of ALA and PBG did not return to pretreatment concentrations for 5 and 11 days, respectively, during which time no more hematin was infused. This suggests that the hematin was not being rapidly converted to bile pigment, as suggested in an earlier study (17), in which hematin injected intravenously in normal subjects was largely accounted for as fecal urobilinogen, in from 2 to 4 days. Also, it has been established that hepatic hemes are rapidly converted to bilirubin. Does the combination of hematin or heme with the aporepressor for ALA synthetase delay its conversion to bile pigment?

The final series of infusions of hematin in alternating amounts of 1000 and 500 mg every other day, produced a gradual, progressive decline in serum PBG for 8 days, but the serum ALA concentrations rose gradually. This increase, occurring in an anuric patient, could result from any of three mechanisms: (a) An increase in $R_l$ (ALA) due to "escape" of ALA synthetase from hematin repression; (b) a decrease in $R_2$ (conversion of ALA to PBG); (c) increase in the rate of net tissue release of ALA into serum. By itself, however, mechanism (a) cannot explain the decline in PBG concentrations concomitant with the increase in ALA, whereas mechanisms (b) and (c) are compatible with both curves (Fig. 1). Mechanism (b) implies an inhibition of ALA dehydrase by hematin (Fig. 3), a phenomenon that has been demonstrated in mammalian liver (19) and erythrocytes (20). In addition, since ALA concentrations were increasing significantly, it is likely that there was less than complete repression of ALA synthetase, even by these large amounts of hematin. It is possible that, as in rats (11), hematin cannot repress all synthesis of ALA, either because there is a single enzyme not subject to complete repression, or because there are two or more routes by which ALA
can be made, the major of which is repressible by hematin, while the minor is not repressed. Thus, the rise in serum ALA concentrations observed during the final series of infusions of hematin could have been a result of a relatively constant, less-than-complete repression of ALA synthetase by hematin and an increasing inhibition of ALA dehydrase as more hematin accumulated in the liver. Data are lacking either to support or refute the above hypothetical mechanism (c).

Three hypotheses as to possible mechanisms of the acute attack of acute intermittent porphyria have previously been discussed (4): (a) A direct adverse effect of porphyrin precursors or unknown derivatives; (b) diminished heme formation with consequent induction of ALA synthetase; (c) a defect outside the heme biosynthetic pathway is linked to the induction of hepatic ALA synthetase and predisposes to nervous system damage. Nothing has been published to support or refute the third hypothesis.

With regard to the first mechanism, the relationship between the increased concentrations of porphyrin precursors in blood and cerebrospinal fluid, and attacks of neurologic dysfunction in patients with acute intermittent porphyria remains unknown. If these substances or derivatives exert an inimical effect on the nervous system, one might expect that an agent capable of lowering profoundly their production rates ought to be of therapeutic value. This, however, could scarcely be tested in this patient as the severe complications, especially the malignant hypertension and uremia, imposed irreversible obstacles to recovery, even had the porphyria been brought into remission as a result of the hematin injections. Also, the period of “treatment” with hematin was relatively brief and had been preceded by severe nervous system dysfunction. Some patients excrete relatively large amounts of PBG and ALA, though asymptomatic for years, suggesting that these precursors are unrelated to the neurologic damage. Yet, it is noteworthy that in two fatal cases of acute intermittent porphyria, the present one and another recently studied (7), the serum PBG concentrations were the highest thus far recorded, much higher in fact than in several cases in remission who regularly excrete considerable amounts of PBG in the urine. Furthermore, the values in the present case, even when lowered after the administration of hematin, were generally much higher than those encountered in cases in remission. It has been reported that both PBG and ALA inhibit neuromuscular transmission (31), PBG more markedly. The significance of these observations in the case of acute intermittent porphyria remains to be determined.

The recent demonstrations of decreased conversion of PBG to porphyrins in acute intermittent porphyria (5–7) raise the question whether the clinical manifestations of the disease might be due to decreased heme synthesis in the nervous system (hypothesis b). If intravenously administered heme were taken up and utilized by the nervous system, it might be of therapeutic value, even though its mode of action differed from its effect in repressing hepatic formation of ALA and PBG. It is also possible that hepatic heme uptake, though correcting the characteristic biochemical abnormalities in the liver, might fail to produce clinical benefit due to failure of uptake of administered heme by the nervous system.

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