Temporal Relation Between a Hepatic Erythropoietic Factor and the Site of Rat Erythropoietin Production

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ABSTRACT

Serum borne hepatic erythropoietic factor (HEF), which can stimulate hepatic erythropoietin (Ep) production in the adult rat, is found at elevated levels in the serum of partially hepatectomized rats and of rats subjected to hepatotoxic injury. It is also detected in sera of patients with liver disease. The purpose of the present study was to determine whether or not HEF activity is increased in the serum of the normal neonatal rat at a time when the liver is the primary site of Ep production. Our results show significantly increased HEF activity in the serum of young rats during the second to fifth weeks of life. Negligible activity was detected in rats over five weeks of age. In the rat, the kidney is reported to begin producing Ep by the third week of life and by the eighth week the kidney is the major site of synthesis with liver production at this age significantly diminished. Thus, our findings show a temporal relation between HEF activity in the serum and the reported transition from liver to kidney production of Ep.

Introduction

Erythropoiesis in mammals is regulated by the hormone erythropoietin (Ep).8,9,14 The primary organ of Ep synthesis in the adult is the kidney8,9,14 whereas in the fetus, Ep is produced primarily by the liver.9,10,33 Studies in the rat have shown that renal Ep synthesis begins during the neonatal period, and Ep production from this source reaches adult levels at about three to four weeks.
of age. In the sheep, the switch of production of Ep from the liver to the kidney is completed by the sixth week after birth. In the adult rat, the liver retains its ability to produce Ep but at a significantly reduced level. In the adult rat subjected to hypoxia, approximately 10 to 15 percent of the total Ep response has been found to be hepatic in origin. Hepatic Ep production may be enhanced by the administration of erythrocyte degradation products, during protein deprivation, following the injection of renin or angiotensin, after the injection of carbon tetrachloride, and by chronic administration of phenylhydrazine.

Our laboratory and others have shown that hepatic Ep production following hypoxia in the rat can be significantly increased if the liver is allowed to regenerate following subtotal hepatectomy. Studies in our laboratory have also shown that under certain stress conditions the hepatic Ep response is under the control of a serum borne factor produced by the liver and named the hepatic erythropoietic factor (HEF). The HEF is an entity derived from the sera of rats following hepatectomy which, when injected into normal rats 18 hr before bilateral nephrectomy and hypoxia, stimulates hepatic Ep production. An HEF-induced Ep response to hypoxia has been observed in rats with hepatic necrosis induced by either acute or chronic administration of carbon tetrachloride or acute injection of acetaminophen.

Titers of HEF are also elevated in the intact rat by chronic administration of phenylhydrazine without exposure to hypoxia. In addition, HEF-induced Ep levels are augmented in normal rats receiving serum from hepatectomized animals. The HEF-induced hepatic Ep mechanism seems to function in humans as well as in animals since increased HEF activity is found in the serum of nephric patients with liver disease or combined kidney and liver disease. Levels of HEF are also higher in anephric patients with liver disease. Evidence has been presented that the kidney inhibits the activity and/or synthesis of the HEF by producing a serum borne renal inhibitory factor.

In the sheep and the rat, a natural transition between liver and kidney production of Ep occurs in the neonatal period. It has been previously established that the serum HEF titer is low in the normal adult rat when the kidney is the primary site of Ep production. The purpose of the present study is to determine whether or not a temporal relation exists between the presence of HEF in the serum of the rat and the transition from liver to kidney production of Ep. A finding of a high titer of HEF in the serum of the neonatal rat at the time when the liver is the main organ of Ep synthesis, and a low titer of HEF in the serum of the adult rat when the kidney assumes the major role in Ep production, would be evidence that HEF may be involved in the normal physiological mechanism regulating the switch of Ep production from liver to kidney.

Material and Methods

Titers of HEF were assayed in serum obtained from normal one to 16 week old Long-Evans rats bred in our laboratory. The rats were fed a diet of Ralston Purina rat chow with tap water ad libitum. Temperature, humidity, and lighting were automatically controlled. A record was kept of the date of birth of each litter to allow an accurate determination of the age of the rats at the time of serum collection. The rats' weight ranged from 18 to 22 g for the one week to 400 g for the 16 week old rats. Serum was obtained by allowing whole blood collected by aortic
exsanguination into sterile, non-toxic, non-pyrogenic syringes to clot at room temperature for 30 min. The blood was then centrifuged in glass tubes at 700 g for 10 min and the supernatant serum removed. The serum collected from the one to four week old animals was obtained from both male and female donors and serum collected from rats older than five weeks from male donors. The serum of 10 to 12 one week old, 10 to 12 two week old, 8 to 10 three week old, 5 to 8 four week old, 4 to 5 five week old, and 3 to 4 six week and older rats were pooled for each HEF assay. Blood was pooled because a volume of blood sufficient for the HEF assay could not be collected from a single young rat (up to five weeks of age). Pooling was done for the older rats (six weeks of age and above) to maintain a similar serum collecting procedure for the assays. Assays were performed each week up to the seventh week of age and on the ninth, 11th, 14th, and 16th week after birth. From three to nine assays of pooled serum were conducted for each of the 11 age groups studied. The number of assays indicate the number of tests conducted for each group.

HEF Assay

The tandem double assay procedure for assaying HEF activity was described previously. Briefly, two ml of serum collected from the one to 16 week old donor rats were injected i.p. into normal male recipient (assay) 200 g rats. The rats were then bilaterally nephrectomized 18 hr after serum administration and after a one hr interval were subjected to six hr of hypobaric hypoxia at 0.4 atm of air. Following this, the rats were exsanguinated and their serum assayed for Ep in the exhypoxic polycythemic mouse.  

EP Assay

BS1 virgin female mice were used for the assay of Ep. The mice were subjected to discontinuous hypoxia for 19 hr per day at 0.4 atm for two weeks. After the cessation of hypoxia, the polycythemic mice were most sensitive to Ep present in the serum collected from the assay rats, since they produced little or no endogenous Ep. No mice with hematocrits of less than 55 percent were utilized in the assay. Each sample point was assayed in three to four mice. The percent $^{59}$Fe uptake into the newly formed erythrocytes of these animals evoked by one ml of test serum was compared to the radioiron uptake into red blood cells of similar mice treated with standards derived from the International Reference Preparation for Ep.  

The rationale for the use of this tandem double assay procedure for assaying for HEF is as follows: HEF is a factor found in the liver which can stimulate Ep production by the liver of a normal animal during hypoxia. Eighteen hr are allowed to elapse between test sample injections and nephrectomy to prime the liver for Ep production. Nephrectomy must be performed so that extrarenal Ep can be detected. Renal extirpation also eliminates the source of a potential inhibitor to HEF, the renal inhibitory factor (RIF). The quantity of hepatic Ep produced by the liver of this first assay animal in response to hypoxia is indicative of the titer of HEF in the test sample. One hr is permitted to elapse between nephrectomy and the onset of hypoxia to allow the rat to recover from the anesthesia.

Serum found to contain HEF activity has been assayed for Ep, and little or no Ep activity was detected. In addition, the possible presence of Ep in the test serum injected into normal recipient rats as a factor in the assay is further pre-
cluded because of the dilution resulting from the injection of a relatively small vol of test serum (2 ml) into the assay rat coupled with the fact that the half life of Ep is only approximately two hr.20

Results

All data are presented in figure 1 as HEF-induced Ep levels per ml of serum in assay rats following the administration of serum from one to 16 week old normal donor rats. The quantity of hepatic Ep produced by the assay rat is indicative of the titer of HEF in the test serum. Thus, an increase in the assay rat hepatic Ep response to hypoxia is indicative of the presence of increased serum HEF activity under these experimental conditions.5,7,22,23,27 No meaningful differences were found between HEF assays conducted on the pooled serum samples collected from the immature rats of similar age and different sexes in the one to four week old age groups. A total of 11 age groups were studied.

As seen in figure 1, the mean HEF-induced hepatic Ep titers were significantly higher (Student's T test; P < 0.05) in the serum of assay rats that were injected with serum of normal two to five week old donor rats as compared to serum collected from assay rats which were administered serum of normal six to 16 week old donors. The mean HEF-induced hepatic Ep titer in the serum of assay rats administered serum of one week old normal donors was non-significantly higher than the mean HEF-induced hepatic Ep titers in assay rats administered serum of animals older than five weeks. This was because only four of the eight assays performed on the serum collected from the one week old donor rats showed increased HEF activity. Since the HEF assays were performed at periodic weekly intervals, it is not known if a specific onset of HEF production in those rats with negligible activity would have occurred between the first and second weeks of age. Assays performed on the serum collected from the 6, 7, 9, 11, 14, and 16 week old donors showed uniformly negligible HEF activity. None of the assays conducted on the serum collected from the rats in these age groups showed elevated HEF activity.

Discussion

The results of this study indicate the presence of elevated levels of HEF activity in the serum of normal rats of up to five weeks of age with no meaningful activity detected in older rats. The mean HEF activity in the serum of one week old rats, although high, was not significantly different from the mean HEF activity in the serum of the older animals owing to negligible HEF activity in 50 percent of the assays. This may be due to the presence of other factors in the serum of the very young animal which are as yet obscure. By the sixth week of age, serum HEF activity is significantly reduced. Our finding of elevated HEF
activity in the serum of the neonatal rat correlates well with the findings of others who have investigated the temporal transition from liver to kidney production of Ep. In this connection, it has been reported that subtotal hepatectomy in 10 day old neonatal rats resulted in almost complete abolition of Ep production in response to hypoxia, whereas nephrectomy had no effect on Ep levels. It has been further shown that the ability of three week old male and female rats to elevate their serum Ep levels in response to hypoxia is significantly depressed following partial hepatectomy and almost completely abolished following partial hepatectomy and nephrectomy. In another study using one week, two week, three week, and eight week old rats it was demonstrated, by subjecting the animals to a combination of hepatectomy and nephrectomy, that the primary site of Ep production during the first two weeks of life is the liver. In this same study it was found that the kidney begins producing Ep by the third week of life and was the major site of Ep synthesis by the eighth week. These findings demonstrating the temporal transition from liver to kidney production of Ep in the rat correlate well with our present study in which significant HEF activity is found in the serum of rats up to the fifth week of life but not in the older animal. Thus, a temporal relation is evident between the presence of HEF activity in the serum and the time of transition from liver to kidney Ep production.

Hepatic erythropoietic factor has been demonstrated in several animal models, including the hepatectomized rat and animals subjected to hepatotoxic injury, as well as in humans with liver disease. Studies have also been undertaken to characterize the nature of HEF and to determine its relationship to other hepatotrophic factors. Hepatic erythropoietic factor was found to be resistant to both proteinase K and pronase treatment, indicating that protein may not be necessary for the action of this molecule. Amicon filtration studies and Sephadex G-15 gel filtration have shown that the mol wt of HEF is approximately 1000 D. This finding differentiates the HEF from other hepatotrophic components present in the serum of animals with regenerating livers. In this regard, several moieties have been identified which induce hepatocellular proliferation, but these are considerably larger molecules, ranging from 10,000 to 50,000 D. The HEF does not appear to be prostanoïd in origin since treatment of HEF stock with antisera to prostaglandins of the E and A series did not significantly alter the HEF activity. Finally, thromboxane, a low molecular weight derivative of unsaturated fatty acids, which is reported to initiate the hepatic regenerative response, requires an additional but as yet uncharacterized component from fetal calf serum. In contrast, HEF induced Ep production is independent of the administration of any exogenous substances. Studies have also been undertaken in our laboratory to identify the hepatic site of HEF production. Present evidence suggests that the hepatic parenchymal cells are the source of HEF which then stimulates Ep production by the Kupffer cells. To date, elevated HEF titers have been found only in the serum of rats and in humans that have been subjected to stress conditions. The importance of the present study is that for the first time significant HEF activity has been found in normal rats at an age when the liver is the primary source of Ep production. By the sixth week of life when the kidney is the primary source of Ep production, HEF activity in the serum is significantly reduced. Therefore, HEF activity in the serum of the young rat is temporally related to the switch from liver to kidney production of Ep. These findings suggest
that HEF may contribute to or be a physiological control mechanism for regulating the normal transition of Ep production from the liver to the kidney.

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