Evidence for a Hepatic-Renal Antagonism in the Production of Hepatic Erythropoietin.*

BRIAN A. NAUGHTON, PH.D.,
BURTON S. DORNFEST, PH.D.,
RICHARD JOHNSON, B.S., MARYANN L. HUIE, B.S.,
and ALBERT S. GORDON, PH.D.

A.S. Gordon Laboratory of Experimental Hematology,
Dept. of Biology, New York University,
New York, N.Y. 10003
Downstate Medical Center, Dept. of Anatomy,
Brooklyn, N.Y. 11217
Hunter College School of Health Sciences,
Dept. of Medical Laboratory Sciences,
New York, N.Y. 10010

ABSTRACT

A tandem perfusion of the liver and kidneys of hepatectomized (hepx) rats was performed to ascertain the precise levels and time of appearance of the previously reported hepatic erythropoietic factor (HEF) and its antagonist, the renal inhibitory factor (RIF). Hepatic erythropoietic factor is produced by the hepx animal and is capable of augmenting the hepatic erythropoietin (Ep) response to hypoxia when administered to normal rats, whereas RIF acts to diminish this Ep response. Renal inhibitory factor does not directly affect Ep but appears to alter its synthesis owing to an inhibition to HEF production and/or action. In the present study, levels of HEF and RIF were determined at different intervals following hepx by in situ perfusion of the livers and kidneys of these animals. Levels of HEF are significantly higher than controls in rats at 24 to 72 hrs post hepx whereas its antagonist, the RIF, is not detectable until between 48 and 72 hrs after hepx. Inhibition of RIF of the hepatic Ep response is nearly total by 96 hrs following surgery. In previous studies, an increase in the hepatic Ep response to hypoxia was noted from 24 to 72 hrs after hepx. Peak hepatic Ep levels occurred at 72 hrs post hepx and declined thereafter, manifesting near normal hepatic Ep levels at 96 hrs after hepx. The present study provides an explanation for these findings by demonstrating that production of Ep by the hepx animal is dependent on the relative levels of a stimulatory (HEF) and an inhibitory (RIF) factor.

* Address reprint requests to: Dr. Brian A. Naughton, A. S. Gordon Lab. of Exptl. Hematology, Dept of Biology, New York University, 100 Washington Square East, New York, NY 10003.
Introduction

Mammalian erythropoiesis is regulated by the hormone erythropoietin (Ep) which is primarily of renal origin in the adult animal. The liver is the most potent Ep producing organ in the fetus and neonate and is also an important extrarenal source of Ep in the adult animal as well, contributing approximately 10 to 15 percent of the total Ep response to hypoxia. Hepatic Ep elaboration after hypoxia was significantly higher in rats with livers regenerating after partial surgical removal or following exposure to toxic agents. Subtotal hepatectomy (hepx) and/or toxic liver injury may confer upon the adult liver the ability to revert to a fetal-like condition. In this regard, the adult regenerating liver not only produces elevated levels of Ep but again becomes a hematopoietic organ, supporting erythropoiesis and granulopoiesis as well. These processes appear to be under humoral control. A serum borne entity, termed the hepatic erythropoietic factor (HEF), is produced after liver removal or chemical injury and is capable of stimulating the hepatic Ep response to hypoxia. Sera from hepx animals exhibit both erythropoietic and hepatotrophic effects when administered to normal rats. Although the former action has been attributed to HEF, a number of growth-promoting substances have been identified in the circulation of animals after hepx, accounting for the hepatotrophic response.

Hepatic erythropoietic factor has been localized in the regenerating liver via an in situ perfusion technique. The synthesis and/or mode of action of HEF is antagonized by a renally-derived moiety, termed the renal inhibitory factor (RIF). The RIF is demonstrable in the renal venous effluent of hepx rats and inhibits the HEF-induced hepatic Ep response to hypoxia. Renal inhibitory factor has no anti-Ep activity per se and titers of this factor in normal rat serum are negligible. Hepatic Ep levels in response to hypoxia are initially diminished after hepx but increase steadily until they peak at 48 to 72 hrs post surgery. They decline thereafter, reaching a nadir at 120 to 144 hrs after hepx. The rise in Ep levels of these hepx animals has been attributed to the action of HEF whereas the declining hepatic Ep response to hypoxia, noted as regeneration progressed from 96 to 144 hrs following hepx, has been attributed to an RIF inhibition of HEF synthesis and/or action. The aim of the present experiment is to study in greater depth the interrelationship of HEF and RIF in the hepatic Ep response to hypoxia.

Materials and Methods

In this study, HEF and RIF levels in normal male Long-Evans rats (210 to 265 g) were compared to those in animals with livers regenerating after subtotal hepatectomy (hepx).

Perfusion

Rats were subjected to a 60 to 80 percent hepx by methods previously described. At 0, 24, 48, 72, or 96 hrs following surgery, the rats were anesthetized with nembutal and a tandem perfusion of the liver and one kidney of each animal was performed using perfluorocarbon, a blood substitute. This compound possesses blood gas carrying capabilities. The rats were placed on a temperature regulated platform maintained at 37.5°C and injected with 0.4 mg of heparin in 0.2 ml of 0.9 percent saline via the femoral vein. The abdomen was opened by a mid-line incision to expose the viscera which were kept moist by periodic application of saline warmed to 37.5°C. A Harvard peristaltic pump was used to perfuse the perfluorcarbon solution (maintained at 37.5°C in a water
bath) through the left kidney and the liver. To prepare the left kidney for perfusion, the aorta was separated from the inferior vena cava below the origin of the left renal vessels. All branches of the aorta and inferior vena cava above and below the origin of the left renal not involved in the circulation of the left kidney were ligated, including the right renal vessels, the left inferior suprarenal vasculature, and the testicular veins. To prepare the liver for perfusion, the common hepatic artery, abdominal segment of the inferior vena cava, and all other vessels not involved in the hepatic circulation were ligated to isolate completely the liver from the systemic circulation. This procedure has been previously described.5

An arterial cannula was then inserted into the aorta and a venous cannula into the inferior vena cava. Both cannulae were advanced to just below the origin of the left renal vessels and sutured in place. The left kidney was perfused at a flow rate of four to five ml per min for three to four min during which time a total of 15 to 19 ml of perfusate was collected. After perfusion of the kidney, the arterial cannula which served as the inflow pathway was inserted into the portal vein and the venous cannula inserted through the right atrium into the thoracic portion of the inferior vena cava and advanced to the juncture of the hepatic vein. The cannulae were sutured in place and the liver was perfused at a rate of five to seven ml per min for 1.5 to three min during which time a total volume of 15 to 19 ml of perfusate was collected. The first one to two ml of perfusate collected from each organ were discarded to eliminate any Ep, HEF, or RIF which might be found in the trapped renal or hepatic blood volume. The rats remained alive throughout the perfusion period.5

The perfusate collected from each rat (three to four animals per group) was centrifuged to remove the cells and the supernatant was assayed for HEF (liver perfusion) and RIF (kidney perfusion). Each data point comprised three to four groups.

ASSAYS

HEF: The tandem double assay procedure for this entity has previously been described.5,16 Briefly, two to four ml of perfusate were injected i.p. into a normal rat which was bilaterally nephrectomized (nephx) 18 hrs later and subjected to six hrs of hypobaric hypoxia at 0.4 atmosphere of air.16 The animals were exsanguinated and their serum assayed for Ep in the exhypoxic polycythemic mouse (described later). Elevated hepatic Ep levels when compared to normal controls are indicative of the presence of HEF. The volume of perfusate administered to each recipient rat was determined by comparing the total volume of perfusate collected from each organ to their blood volumes, as calculated from individual organ weights, using the method of Mejia.14

RIF: This is also a tandem double assay procedure.18 Assay rats were hepx and 42 hrs later were nephx and injected with test material directly into the renal vein. At six hrs following injection, the animals were subjected to hypoxia (0.4 atmosphere per 6 hrs.). After hypoxia, these rats were exsanguinated via the abdominal aorta, and their serum assayed for Ep. A diminished hepatic Ep response to hypoxia in these animals, when compared to normal rat perfusate injected controls, is indicative of the presence of RIF.18 As with the HEF assay, the initial injection volume varied depending on the dilution factor calculated using renal blood volume (determined on a basis of organ weight14) and the total amount of perfusate collected from each kidney.

The rationale for the use of this animal model is as follows: Hepx rats produce HEF which stimulates hepatic Ep pro-
duction. Since the Ep-inducing potential of HEF is greatest in the absence of the kidneys, the rats are nephrx prior to the injection of test material. This insures maximum activity of HEF by eliminating a possible source of endogenous inhibitor to this factor, thus permitting a more accurate appraisal of inhibitory activity in the test material. In addition, six hrs are allowed to elapse between nephrx and test serum injection, and hypoxia. It is believed that this provides a more sensitive test for Ep inhibition since it has been shown that Ep levels diminish as the time between nephrx and the onset of hypoxia increases.

The proposed interrelation between HEF, RIF, and hepatic Ep production is depicted in figure 1.

Ep: Perfusate of kidney and liver for each experimental and control rat was assayed for Ep in the exhypoxic polycythemic mouse. The assay mice were subjected to discontinuous hypoxia for 19 hrs per day at 0.4 atmosphere for two wks. After the cessation of hypoxia, these polycythemic mice are most sensitive to Ep in the test material, since they produce little or no endogenous Ep. The percent of RBC $^{59}$Fe uptake in these animals evoked by one ml of test material was compared to the uptake in similar mice treated with standards derived from the International Reference Preparation (IRP) for Ep$. Log-dose regression lines for calculation of slope values have been consistently parallel, with few exceptions, over the years in which the assay has been employed. The reader is referred to Camiscoli and Gordon4 for a detailed discussion of the development and use of this assay with a description of inter-series and in-series variations as well as the details of the statistical analysis of the data.

Results

All data are found in figure 2, which depicts Ep levels per ml of serum in recipient rats administered serum containing either HEF or RIF activity. Normal rat serum controls are indicated by the broken line. Significant elevation in recipient rat hepatic Ep response is

![Figure 1](image-url)

**Figure 1.** The proposed interrelation between hepatic erythropoietic factor and renal inhibitory factor and hepatic erythropoietin production.
Indicative of the presence of HEF, whereas substantial diminution of this parameter indicates RIF activity. Hepatic erythropoietic factor-induced hepatic Ep titers become significantly elevated when compared to controls between 24 and 48 hrs after hepx of the donor rat and achieve their peak levels at 48 hrs post hepx (figure 2) (p < 0.05). These results are in agreement with the findings of Liu et al who used the doubly hepx animal as an HEF donor. Mean HEF-induced hepatic Ep titers are lower 72 hrs after hepx of the donor as compared to 48 hrs, although this difference is not statistically significant. The HEF-induced hepatic Ep levels then drop drastically, nearing control levels at 96 hrs after hepx of the donor rat. This is consistent with previous reports on the HEF effect. The RIF (as indicated by a drop in the recipient rat hepatic Ep response to hypoxia) is not detectable until 48 to 72 hrs following hepx of the donor rat (p < 0.05). The degree of inhibition of the recipient’s hepatic Ep response is nearly total by 96 hrs post hepx of the donor animal (figure 2) (p < 0.02).

**Discussion**

The initial observation that hepx could induce an augmented hepatic Ep response to hypoxia spawned further studies on the mechanism(s) underlying this effect, including the present report. Hepatic regeneration is acknowledged to be a humorally-mediated phenomenon and so, it appears, is Ep synthesis by the regenerating liver. A hepatic-renal antagonism regulating Ep production by the regenerating liver has been hypothesized: the activity of a hepatic Ep stimulatory factor (HEF), which is produced after hepx, is inhibited by a second entity, released by the kidney (RIF). The interrelationship of these factors is depicted in figure 1. Renal inhibition of hepatic Ep elaboration is not attributable to removal of the HEF via normal kidney excretory function, since this activity can be transferred with serum. In addition, RIF does not have any anti-Ep activity, but appears to act directly on HEF synthesis and/or effect.

In the present study, HEF synthesis
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precedes that of RIF by approximately 24 hrs and apparently stimulates its production (figure 2). Significant titers of serum HEF are noted between 24 and 72 hrs after hepx, with a peak at 48 hrs post surgery. In contrast, inhibition of hepatic Ep synthesis by the RIF is not significant until 72 hr following hepx (figure 2) (p < 0.05). By 96 hrs after hepx, RIF elaboration by the kidney is substantial and completely abolishes any hepatic Ep stimulatory effect owing to HEF (figure 2) (p < 0.02). The RIF assay involves the use of a rat which is nephrx and injected with test solution at 42 hrs after hepx. The six hrs lag time between injection of material for RIF assay and onset of hypoxia (0.4 atmosphere per 6 hrs) places the animal at 48 hrs post hepx, where highest HEF titers are observed (figure 2). It is against these peak HEF levels that RIF activity is measured. Serum of normal animals contains neither HEF nor RIF, at least not in quantities detectable by our current assay system. Since HEF and hepatic Ep levels are essentially normal by 96 hr after hepx, RIF titers probably diminish shortly thereafter, although RIF levels were not measured in animals after 96 hrs post hepx in this study. The rapid rise and subsequent decline of hepatic Ep levels after hepx can be explained, in part, by these findings. After partial liver removal or following hepatic injury owing to toxic agents, regeneration commences and HEF is produced in increasing quantities, dependent on the mass of active hepatic cells. This entity then induces synthesis and/or release of Ep from the liver. Hepatic Ep production by the hepx animal parallels the rate of liver regeneration. Hepatic erythropoietic factor appears to stimulate the production of RIF by the kidney, which increase until they eventually block HEF synthesis and/or action and abolish the hepatic Ep response to hypoxia.

Concomitant with these changes is the appearance of hematopoietic foci in the regenerating liver, which are either erythropoietic or granulopoietic in nature. This reversion to a fetal-like condition may be brought about by an alteration in the hepatic microenvironment incurred by substantial tissue removal or toxic injury. Extramedullary hematopoiesis in the livers of adult animals has also been reported after phenylhydrazine treatment, carbon tetrachloride administration, methylcellulose injection, and during Friend virus infection. Treatment with these chemical and infectious agents results in substantial alterations in hepatic ultrastructure. The liver is the most important Ep producing organ in the fetus, but this function becomes primarily renal shortly after birth in most mammals. As the kidney assumes this function, hepatic Ep elaboration diminishes and the liver loses its ability to support erythropoiesis. Since HEF/RIF control of the hepatic Ep response to hypoxia is operable in the hepx animal, whose liver has many of the characteristics of the fetal liver, a similar mechanism may regulate the transition from liver to kidney Ep production, which occurs in the neonatal mammal. In this regard, the cessation of hepatic Ep production and erythropoiesis may be contingent on the elaboration of RIF by the kidney, which then takes over Ep synthesis as the circulating levels of hepatic Ep diminish.

In summary, the regulation of Ep production in the hepx animal by a hepatic Ep stimulator (HEF) and an inhibitor to this entity (RIF) derived from the kidney was studied. Appearance of HEF after hepx precedes that of RIF by approximately 24 hrs and apparently stimulates its production. By 96 hrs after hepx, RIF is present in sufficient quantities to reduce the Ep response to hypoxia by the regenerating liver to the levels found in normal livers. A hypothesis is put forth that similar HEF/RIF control mecha-
isms regulate the transition from hepatic to renal Ep production which occurs in the neonatal mammal.

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References


