Erythropoietin Production in Response to Anemia or Hypoxia in the Newborn Rat

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Erythropoietin production in response to hypoxic-hypoxia is markedly reduced in the newborn when compared to the adult rat. This response improves steadily with age and reaches adult values at about 4 wk. When animals of the same age are stimulated with anemic-hypoxia, considerably higher levels of erythropoietin are found. The erythropoietin level is proportional to the degree of anemia and independent of the age of the animal. Extraction of erythropoietin from tissue homogenates revealed a parallelism between the plasma and kidney erythropoietin content, while no erythropoietin could be extracted from liver tissue at any age. The lack of response to hypoxia in the newborn appears to be related to the high hemoglobin oxygen affinity during the neonatal period, which facilitates oxygen loading. Newborn rats have a very low intraerythrocytic concentration of 2-3 DPG and a marked shift to the left in the oxygen hemoglobin dissociation curve that slowly increases to adult values at 4 wk of age. The response to anemia on the other hand, appears to be normal and not affected by age or by hemoglobin oxygen affinity. These studies suggest that the newborn rat, when properly stimulated, is able to produce normal amounts of erythropoietin, most likely renal in origin.

It appears firmly established that the kidney is the main source of erythropoietin (Epo) in adult mammals. During fetal life, however, extrarenal sites, most likely the liver, appear to play an important role in the production of erythropoietin. In the sheep fetus, Zanjani et al. have shown a switch from liver to kidney, which occurs around the time of birth. In rats, Gruber et al., utilizing immunofluorescent techniques, have claimed that there is a similar switch from liver to kidney production of erythropoietin at around 3 wk of age. Earlier studies by Carmena et al. had shown that the newborn rats have a marked reduction in their erythropoietin production in response to hypoxia. In these animals, bilateral nephrectomy results in only a 50% loss of the plasma erythropoietin levels, suggesting an extrarenal origin of a significant portion of the already low erythropoietin production. The ability to produce erythropoietin in response to hypoxia gradually improves during the neonatal period, and adult levels are reached at around 3–4 wk of age. Concomitantly to these changes in erythropoietin production, the moderate anemia of newborn rats becomes more severe, reaching a nadir at 2–3 wk of age, after which the red cell count increases towards normal.

The recent development of techniques for extraction of erythropoietin from tissue homogenates has made it possible to examine more directly the question of renal erythropoietin production during the newborn period in the rat. The studies reported here on the erythropoietin levels in the plasma, kidney, and liver of rats at different ages exposed either to hypoxia or anemia suggest that the kidney of the newborn rat is fully capable of normal erythropoietin production and that the decreased erythropoietin response to hypoxia is caused by low hemoglobin oxygen affinity rather than a delayed liver to kidney switch.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats were used throughout the study. Virgin female rats were mated for two nights with males of the same strain. The pregnant mother, fetuses, and offspring were used in the experiments described. Hypoxia was induced by placing the animals in a steel chamber connected with a vacuum pump and keeping them at 0.4 torr for 6 hr. Immediately after hypoxia, blood was obtained either by aortic puncture or decapitation under a light ether anesthesia, and tissues were removed and processed for erythropoietin assay. Anemia was induced by a subcutaneous injection of 60 mg/kg of freshly prepared and neutralized phenylhydrazine. Twenty-four hours later, blood and tissues were obtained as described above.

Erythropoietin Assay

Erythropoietin was measured by a bioassay utilizing hypertransfused polycythemic mice as previously described. The lower level of the sensitivity of the assay is 50 mU of erythropoietin, with a linear dose–response curve up to 1000 mU. Plasmas from fetuses and newborn animals were pooled in order to obtain enough material for the assay. In larger animals, plasma from single donors was utilized.

Tissue Erythropoietin

Tissue homogenates were prepared according to a modification of the technique described by Fried et al. In summary, the organs were rapidly removed after exanguination of the animal and processed throughout at 4°C. They were minced with scissors and carefully washed with cold 0.1 M phosphate saline buffer at pH 7.6 (PBS). They were then homogenized in a rotary blade homogenizer in a mixture of 50% normal rat plasma of 50% PBS. The homogenate...
was centrifuged at 15,000 g for 30 min and the supernatant collected for assay. Supernatant equivalents of 0.4 g of original tissue were injected subcutaneously once a day for 2 days to each polycythemic assay mouse and the erythropoietin content measured by a routine assay. In control studies it had been established that rat erythropoietin added to normal liver or kidney tissue and processed as above resulted in about a 30% loss of activity, although no significant difference in recovery was observed between liver and kidney homogenates.

Erythropoiesis was assessed by the uptake of radioactive iron in peripheral red blood cells 24 hr after the intraperitoneal injection of 1 μCi of ⁵⁹Fe. A blood volume of 6% of body weight was utilized for all animals regardless of age. Hematocrit, hemoglobin, and reticulocyte count were determined by routine techniques. Intracellular 2,3-diphosphoglycerate (2,3-DPG) was measured in washed red cells utilizing a Sigma Kit (Sigma, St. Louis, Mo.). P₅₀ determinations were carried out in Dr. Toshio Asakura’s laboratory at the University of Pennsylvania utilizing microblood samples and continuous recording.

RESULTS

Growth and Erythropoiesis in the Newborn Rat

Figure 1 shows the presence of a linear increase in weight with an almost tenfold increase in weight during the first 4 wk of life. The rats are born anemic with a hematocrit around 32%. After birth, there is a gradual decrease in hematocrit, with a nadir of hematocrit of about 22% between the second and third weeks of age. Thereafter, there is a steady increase to adult levels. The reticulocyte count in the newborn is very high, around 80%, but rapidly decreases to a steady level of about 10%–15%. This decrease in reticulocyte count, however, is not associated with a change in the rate of erythropoiesis as measured by the 24-hr incorporation of ⁵⁹Fe in red cells.

Erythropoietin Production After Hypoxia

Figure 2A shows the erythropoietin content of plasma, kidney, and liver of 18–19 day-gestation fetuses of mothers subjected to hypoxia for 6 hr and of rats from the ages of 3 days to adulthood similarly exposed to 6-hr hypoxia. As can be seen, the erythropoietin concentration in plasma is low in the fetuses and newborn animals, but shows a steady increase reaching adult values at about 4 wk of age. The content of erythropoietin in kidney homogenates follows a similar pattern, with almost no detectable levels in the fetus and a steady increase up to 4 wk of age. No erythropoietin could be detected at any age in the liver homogenates.

Erythropoietin Production After Phenylhydrazine

Figure 2B shows the erythropoietin level of plasma, kidney, and liver in response to an injection of phenylhydrazine (60 mg/kg). As can be seen, the plasma level of erythropoietin in 2-day-old rats was high, reaching a peak around day 18 and then decreasing to adult values. Erythropoietin content of kidney homogenates again followed the pattern of the plasma erythropoietin. No erythropoietin was detected in the liver homogenates. In evaluating this response to phenylhydrazine, it is important to realize that the degree of
anemia obtained with the same dose of phenylhydrazine differed at different ages. This is due to the fact that, as shown in Fig. 1, newborn animals have a low hematocrit, and this hematocrit drops even lower, reaching a nadir at about 3 wk of age, at the same time as the erythropoietin production is maximal. In Fig. 3, the plasma erythropoietin level of individual samples obtained throughout this study is correlated with the hematocrits of the animals. It is clear that the plasma erythropoietin titers follow the expected inverse correlation with hematocrit and appear to be independent of the age of the animal.

**Hemoglobin Oxygen Affinity of the Newborn Rat**

The results listed above suggests that young rats do not respond to hypoxia as well as they respond to anemia. To explore the reasons for this difference, hemoglobin oxygen affinity at different ages were measured. Figure 4 shows that the levels of 2, 3-DPG are barely detectable in fetal and newborn animals, but increase steadily until adult levels are reached at around 3–4 wk of age. The changes of 2, 3-DPG with age are accompanied by a corresponding increase in P50 from values of 24 mm Hg at day 2 to adult values of 38 mm Hg at day 28.

**DISCUSSION**

The early studies by Carmena et al., which showed that the markedly decreased erythropoietin response to hypoxia in a newborn rat is only partially abolished by bilateral nephrectomy, have suggested that the newborn kidney is only minimally involved in erythropoietin production. The studies reported here confirm that the erythropoietin production in fetuses at term and neonates exposed to hypoxia is much less than that of adult animals. However, the response to hypoxia improves steadily with age and reaches adult values at around 3–4 wk. When rats of the same ages were
Utilizing the recently described technique for erythropoietin extraction from tissue homogenates, a parallelism was found between plasma and kidney erythropoietin content at all gestational ages. These results suggest that the kidney plays the same role in erythropoietin production in the newborn rat as it plays in the adult animal. The extrarenal component of erythropoietin production in newborn animals could not be evaluated adequately, since nephrectomized animals were not studied. However, tissue homogenates of livers failed to reveal significant amounts of erythropoietin both in the newborn and adult animals. In the adult, it can be explained as consequence of the lack of sensitivity of the erythropoietin bioassay. Only about 15% of total erythropoietin production in the rat is extrarenal and presumably derived from the liver, which weighs about 6 times as much as the 2 kidneys. Consequently, the concentration of erythropoietin in the 0.4 g used for assay should be about 1/30th as much as the concentration of 0.4 g of kidneys. In adult rats exposed to phenyldrazine, 0.4 of kidney contain about 150 mU and 1/30th would be 5 mU, an amount not detectable by a bioassay with a lower sensitivity of 50 mU. This lack of sensitivity would also make it impossible to measure the hepatic content in newborn rats. Even at 3 wk, when the erythropoietin content in 0.4 g of kidney reached 1800 mU, the hepatic content, if the distribution was the same as an adult's, would have been a barely measurable 50 mU. If the hepatic component of erythropoietin production in rats was greater in the newborn period than in the adults, erythropoietin should have been demonstrable in liver homogenates. Since this was not the case, it would appear that neonatal erythropoietin production is not predominantly extrarenal, but as in adults, predominantly renal.

The lack of erythropoietin response to hypoxia in the newborn period appears to be related to hemoglobin oxygen affinity. Oxygen transport across the placenta...
is augmented in mammals by the presence of a higher oxygen affinity of fetal than adult hemoglobin. This is accomplished either by the production of a structurally different fetal hemoglobin or by a low intraerythrocytic concentration of 2,3-DPG. In the rat, the latter mechanism prevails and the newborn animals were found to have an almost undetectable level of 2,3-DPG. This was accompanied by a marked increase in oxygen affinity, which steadily reached adult values as 2,3-DPG concentration became normal at around 3–4 wk of age. Since high oxygen affinity facilitates the loading of blood with oxygen at low ambient oxygen pressure, this would explain that newborn rats have less tissue hypoxia and in turn less erythropoietin production when exposed to hypoxia than adult animals. A similar enhanced facility to tolerate hypoxia is found in llamas with high oxygen affinity hemoglobins and in a family with a high oxygen affinity hemoglobinopathy.

As described earlier by Garcia and confirmed in this study, newborn rats are born anemic and their hematocrit decreases further during the first 2–3 wk of life. The presence of an effective renal mechanism for erythropoietin production in response to anemia suggests that this anemia is probably not due to impaired erythropoietin production. In fact, the iron incorporation studies confirm a very active rate of red cell production throughout the first 30 days of life. The most likely explanation is that the newborn rats develop a dilution anemia during the early period of high growth rate and rapid expansion of blood volume.

ACKNOWLEDGMENT

We are grateful to Dr. T. Asakura for his help in the determination of P50 in the newborn rats. We also thank E. Brown and D. Likens for their help in the preparation of this manuscript and Jean Hickey for her technical assistance.

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