Regulation of Erythropoiesis in Suckling Rabbits with and without Postnatal Anemia: Partial Suppression of Production/Release of Erythropoiesis Stimulating Factor(s) by Iron Supplements

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ABSTRACT. The postnatal anemia in rabbits is accompanied by a marked rise in the plasma erythropoiesis stimulating factor(s) (ESF). The purpose of this study was to establish whether the increase in plasma ESF is only related to the anemia, or whether other mechanisms also are involved. Two matched groups of rabbits were studied from the 15th to the 36th day after birth. One group received iron parenterally and had no postnatal fall in hemoglobin concentration (Hb), the other developed the usual anemia. In both groups plasma ESF was undetectable on the 15th day, and also on the 22nd day, despite a marked fall in Hb in the untreated group and rise in the iron-treated group. Thereafter plasma ESF showed a slight, continuous rise in the nonanemic rabbits, in contrast to a marked, transient rise with maximum on the 29th day in the untreated group. On the 36th day there was no difference between the groups. In the iron-treated group the reticulocyte production rate remained unchanged, while the Hb mass rose continuously. In the untreated animals there was an initial decline in reticulocyte production rate, while Hb mass showed a slight increase. From the 29th day, however, reticulocyte production rate rose to the same level as in the iron-treated group and Hb mass rose markedly. In conclusion, the rise in plasma ESF during the postnatal anemia in rabbits is only in part related to the low Hb. Hypoxia-independent mechanisms, probably related to the growth and maturation per se also are involved. The lack of erythropoietic response to the rise in plasma ESF is due to lack of available iron. (*Pediatr Res* 21: 1-4, 1987)

Abbreviations

ESF, activity of erythropoiesis stimulating factor(s)
Hb, whole blood hemoglobin concentration
Hct, hematocrit
MCHC, mean corpuscular hemoglobin concentration
Rt, reticulocyte count
Rt prod, reticulocyte mass production rate
Hb mass, total hemoglobin mass

A postnatal fall in Hb is common to most mammals and is termed the early anemia. In humans, the anemia is most severe in the premature infant. The mechanisms of this drop in Hb are poorly understood. Comparative studies may give information which can give rise to new hypothesis in the human. The erythropoiesis in adult mammals is regulated mainly through erythropoietin in response to variations in renal tissue O₂ tension (1), but other factors may also be involved (2). The tissue O₂-dependent mechanism seems to be established already during fetal life (3-6), but several studies indicate that the regulation of erythropoiesis in newborn animals differs from that which is observed in the adult (4, 7-13).

Previous studies have shown that plasma ESF in young rabbits is very low during the early phase of the postnatal fall in Hb, but increases markedly during the 3rd and 4th wk after birth (10). This may suggest that the regions governing the erythropoietin production/release become increasingly sensitive to low Hb and tissue O₂ tension during this period (10). The possibility also exists that the increase in plasma ESF only in part is due to hypoxic stimulation, and that other, hypoxia-independent mechanisms play a role for the regulation of erythropoiesis at this age. The fact that the postnatal fall in Hb in rabbits can be avoided by parenteral iron supplements [14, 15] makes it possible to distinguish between anemic-hypoxia dependent and independent regulating mechanisms.

The purpose of this study was thus to establish whether the rise in plasma ESF during the postnatal anemia in rabbits is due to the low Hb and the possible tendency to fall in tissue O₂ tension only, or if hypoxia-independent mechanisms also are involved. To accomplish this, plasma ESF, Rt, Hb, and Hct were determined from the 15th to the 36th day after birth in two matched groups of rapidly growing young rabbits. One group received iron parenterally and thus avoided the postnatal fall in Hb. The other group received no supplement and thus demonstrated ordinary development. In order to get a quantitative measure of the changes in erythropoietic activity throughout the study, Rt prod and Hb mass were estimated.

EXPERIMENTAL PROCEDURE

General. To secure a marked fall in Hb, relatively small litters of rapidly growing animals were used (14). Anesthesia and blood sampling were performed as previously described (16). The animals remained with the mother throughout the observation period, but had the choice of food pellets from the age of 21 days.

Determination of relationship between plasma ESF, Hb, and

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Rt in iron-treated and untreated young rabbits. Two litters, each consisting of six suckling Chinchilla rabbits, were used for the study. At the start of the experiment, 15 days after birth, all animals were weighed and 1.6-ml blood samples were withdrawn for analysis of plasma ESF, Hb, Hct, and Rt, and calculation of MCHC, Rt prod and Hb mass. Thereafter three animals from each litter were chosen at random and given intramuscular injections of 5 mg of Fe^{3+} (Jectofer, ASTRA) on the 16th, 18th, 20th, 23th, 24th, 26th, 27th, 31th, 32nd, and 34th days after birth. The remaining animals served as controls. Weighing and blood sampling were repeated when the animals were 22, 25, 29, and 36 days old. The young rabbits tolerated the blood sampling and injections very well. In the course of the first four blood lettings in total 7 ml blood was removed, corresponding to an Fe loss of about 2.0 mg in the untreated and 2.8 mg in the iron-treated group.

ANALYTICAL PROCEDURES

Standard methods. Hb, Hct, and Rt were determined as previously described (10, 15).

Determination of ESF. Plasma ESF was determined by a modification of the cell culture assay for erythropoietin described by Hågå and Falkanger (17), using hepatic erythroid colony forming cells from newborn WLO-mice (18). The assay reflects the net activity of erythropoietin and other stimulatory (2) and possible inhibitory factors. The number of erythroid colonies formed is proportional to this activity in a log dose fashion. The same batch of standard erythropoietin (Sheep plasma erythropoietin step III, Connaught Laboratories, Canada) was used for standardization of the assay and adjustment for interassay variations throughout the study (18). ESF in 24 control cultures with no or 50 mU/ml standard erythropoietin added, were 50 ± 9 and 788 ± 30 colonies/ 0.5×10^5 cells, respectively. The ESF determinations were done in three separate assays, each sample was treated only once.

Calculations. Hb mass was estimated from the body weight and Hb, assuming that the blood volume was 7% of the body weight (9). This percentage seems to decrease slightly with increasing age (19) and thus the Hb mass probably tends to be slightly overestimated during the last part of the observation period, as compared with the initial part. Corrections were made for the influence of blood letting. Rt prod was estimated from the body weight, Hct, and Rt, assuming that the blood volume was 7% of the body weight and that the reticulocytes on an average remain recognizable in the blood for 48 h (20).

STATISTICS

The level of significance of differences between means was determined in accordance with Student's *t* test (for paired observations within groups and for unpaired observations between groups). All observations are given as mean $\pm$ SEM.

RESULTS

The growth was the same in the untreated and the iron-treated rabbits, the weights being 201 ± 27 and 208 ± 27 g on the 15th day, and 458 ± 65 and 466 ± 78 g on the 35th day, respectively ($p > 0.05$).

The untreated group (Fig. 1) showed a marked fall in Hb from the 15th to the 29th day ($p < 0.001$), and thereafter a marked rise to slightly below the initial level on the 36th day. This is the usual development in rabbits at this age (10, 16). In contrast, Hb in the iron-treated group remained essentially unchanged from the 15th to the 29th day, but showed a slight decrease from the 29th to the 36th day ($p < 0.01$). Initially, on the 15th day, both groups showed plasma ESF levels indistinguishable from observations in nonstimulated control cultures ($p > 0.05$). This also was the case on the 22nd day, despite markedly lower Hb in the

Fig. 1. Hb, ESF, and age in two groups of young rabbits with (O) and without (●) extra iron. The vertical bars designate time of iron supplements (mean values $\pm$ SEM).

Fig. 2. ESF and Hb on the 15th (O) and 29th day (●), in young rabbits with (O—●) and without (O---●) extra iron.

untreated animals. Thereafter plasma ESF in the iron-treated group showed a slight, continuous rise, whereas plasma ESF in the untreated group showed a marked, transient rise, reaching maximum on the 29th day, around nadir of the anemia. On the 36th day, however, there was no difference between plasma ESF of the two groups ($p > 0.05$), the level being slightly above the level on the 22nd day ($p < 0.05$). Comparing the individual changes in Hb and plasma ESF from the 15th to the 29th day (Fig. 2), it appears that the untreated animals, all with a marked fall in Hb, showed a marked rise in plasma ESF. A substantial rise in plasma ESF was, however, also observed in some of the

Table 1. *Rt, Rt prod, Hct, and MCHC in two groups of young rabbits, one without iron supplementation and one given iron intramuscularly 10 times, from the 16th to the 34th day postnatally*

Age (day)		Untreated				Iron-treated			
		Rt (%)	Rt prod (ml/day)	Hct (%)	MCHC (g/dl)	Rt (%)	Rt prod (ml/day)	Hct (%)	MCHC (g/dl)
15	Mean	9.3	0.27	42.1	26.0	11.0	0.33	42.3	26.0
	SEM	1.0	0.02	0.4	0.3	0.9	0.03	1.5	0.3
22	Mean	4.3	0.14	31.2	27.7	8.9	0.39	42.9	27.3
	SEM	0.7	0.03	1.3	0.4	0.7	0.06	1.3	0.4
25	Mean	4.5	0.14	26.2	27.6	8.1	0.35	40.8	28.2
	SEM	0.7	0.03	1.2	0.3	1.6	0.05	0.7	0.2
29	Mean	9.6	0.30	25.1	26.7	5.7	0.29	39.8	28.5
	SEM	0.9	0.03	1.5	0.6	0.6	0.04	1.3	0.2
36	Mean	4.8	0.26	35.3	26.7	3.4	0.28	35.9	28.6
	SEM	1.1	0.06	1.7	0.3	1.8	0.17	0.9	0.2

iron-treated animals without any marked fall in Hb, and also in animals showing increasing Hb.

Rt was relatively high on the 15th day in both groups (Table 1). There was a gradual decline to a low level on the 36th day in the iron-treated group. The untreated group showed a marked fall to low values on the 22nd and 25th day. Thereafter, following the rise in plasma ESF, Rt increased markedly. On the 36th day there was no difference between Rt of the two groups. Correspondingly, the estimated Rt prod (Table 1) remained essentially the same throughout the observation period in the iron-treated group, whereas the untreated group showed a slight decline from the 15th to the 22nd and 25th day, but increased to the same level as the iron-treated on the 29th and 36th day. The changes in MCHC (Table 1) for the most part reflect the variation in Rt.

At the start of the study the estimated Hb mass was the same in the untreated and the iron-treated group, being 1.57 ± 0.21 and 1.58 ± 0.18 g, respectively ($p > 0.05$). Thereafter the Hb mass in the iron-treated animals rose continuously to 4.17 ± 0.55 g on the 36th day. In the untreated group the Hb mass showed a very modest increase to 2.18 ± 0.27 g on the 29th day, and thereafter a relatively steep rise to 3.63 ± 0.52 g on the 36th day.

DISCUSSION

Previous studies have shown that the marked postnatal rise in erythrocyte 2,3-DPG and blood PO_2 50% in rabbit is independent of Hb (15, 16). Thus, the O_2 delivery capacity of the blood in the iron-treated rabbits must have increased markedly in the course of the observation period (15). Accordingly, the slight rise in plasma ESF in these animals (Fig. 1) cannot be ascribed to reduction of tissue O_2 tension in the regions regulating erythropoietin production/release (1). Thus the plasma ESF and erythropoiesis increased in an organism with a higher than normal O_2 supply.

Since the animals were growing rapidly at this time, it is reasonable to speculate that factors which influence growth in general also may stimulate erythropoiesis, erythropoietin production, or act synergistically with erythropoietin. Previous observations may also be interpreted this way. In mice, plasma ESF remains elevated as long as the mice are growing (11). Further, hypertransfusion of rapidly growing young mice does not totally suppress the production/release of erythropoiesis stimulating factor(s) (13). This is also in accordance with observations in the human. In premature infants there is a positive correlation between plasma ESF and weight gain, but no correlation between

plasma ESF and Hb (21). It should also be noted that it has been shown *in vitro* that growth hormone potentiates the effect of erythropoietin (22) and that insulin-like growth factor I stimulates the growth of murine erythroid colony-forming cells independent of erythropoietin (23).

In the iron-treated group there was a slight decrease in Hb from the 29th to the 36th day, despite continued iron supplementation and rise in plasma ESF. This observation supports the view that a change in the regulation of erythropoiesis takes place at this age, coinciding with weaning (14).

The fact that plasma ESF in the untreated animals was the same as in the iron-treated on the 22nd day, despite considerably lower Hb, but was markedly higher on the 25th and 29th day, show that hypoxic stimulation only occurred at the nadir of the anemia. This may indicate that the marked rise in 2,3-DPG and the consequent shift of the O_2 Hb dissociation curve to the right which regularly take place at this age (10, 15), were sufficient to compensate for the fall in Hb and prevent tissue hypoxia on the 22nd day, but not around the nadir of the anemia. However, the difference in estimated O_2 delivery capacity of the blood from the 22nd and to the 29th day was probably small, and the marked increase in plasma ESF may also reflect that the regions governing erythropoietin production/release became increasingly sensitive to low O_2 tension at this stage of development (10). The rise in plasma ESF could also be due to shift from extrarenal to renal erythropoietin production (6, 24, 25).

In the iron-treated animals Rt showed a marked decrease from the 15th to the 36th d (Table 1) despite the concomitant moderate rise in plasma ESF (Fig. 1). This could indicate that the *in vivo* sensitivity of the erythropoietic progenitor cell(s) to the erythropoiesis stimulating factor(s) decreased during the observation period. This is in accordance with observations in mice, showing that the *in vitro* sensitivity to erythropoietin of the erythroid colony-forming cell decreases during the period of postnatal anemia (26). The data may also suggest that unknown inhibitory factors and/or substrate limitations restricted the erythropoiesis. On the other hand, the simultaneous occurrence of a constant Rt prod and a rising Hb mass probably reflects that the erythrocyte population at this age almost totally consists of young cells. Thus, the production of erythrocytes markedly exceeds the destruction of old cells, even at moderate reticulocytosis.

In the untreated animals the estimated Rt prod initially showed a definite decrease, despite rising plasma ESF (Fig. 1). Further, the estimated Hb mass showed a very modest rise only, until the age of 4 wk in the untreated group whereas it rose continuously in the iron-treated group. These observations are in accordance with previous observations, indicating that the postnatal anemia

in rabbits is due to lack of available iron and/or other nutritional factors, which is relieved during weaning (10, 14, 15).

The presented data show that the rise in plasma ESF during the postnatal anemia in rabbits is due only in part to the low Hb. They indicate that hypoxia-independent mechanisms also are involved in the regulation of the production/release of erythropoiesis-stimulating factor(s) at this stage of development. In accordance with observations in other species, these mechanisms could be related to the growth and maturation per se (6, 11, 13, 21, 24, 25).

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