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Erythropoiesis, serum erythropoietin, and serum IGF-I in rats during accelerated growth

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Abstract In this study we have examined the correlation between activity of erythropoiesis and serum concentrations of erythropoietin and insulin-like growth factor I in male and female rats during accelerated growth (day 30-90). We found that fractional incorporation of iron into newly formed red blood cells was linearily correlated with body weight gain. Total iron incorporation into newly formed red blood cells reflecting total daily red cell formation increased almost linearily between day 25 and 80 after birth in both sexes. While serum erythropoietin concentrations decreased in the time interval investigated (25-120 days), serum IGF-I levels increased in both sexes between day 25 and 55. In this period, individual values of total iron incorporation into red blood cells and serum IGF-I concentrations were linearily correlated. Our observations support the concept that IGF-I rather than erythropoietin modulates erythropoiesis during accelerated growth and thus manages a proportional increase in body mass and oxygen transport capacity.

It is well established that red cell mass increases in strict proportion to the body mass during the growth period of mammals (1). This proportionality is physiologically important because it ensures that increased oxygen consumption is matched by an increased oxygen transport capacity. However, the way by which this adaptation is brought about is unknown. Since erythropoiesis in the adult is primarily controlled by erythropoietin (2), one could assume that this hormone is of major importance also within this growth process. Another candidate for the adapation is insulin-like growth factor I. IGF-I is considered to mediate the growth promoting activities of growth hormone (3) and thus to govern growth of the organism. IGF-I has been found to enhance the proliferation of erythroid precursors in vivo (4) and in vitro (5-8). Recent evidence indicates that application of IGF-I to hypophysectomized rats leads to a proportional stimulation of erythropoiesis and body growth (9). The relation between the rate of erythropoiesis and serum levels of IGF-I and erythropoietin in intact animals during the growth period is unknown. In order to distinguish possible roles of IGF-I and erythropoietin in the regulation of erythropoiesis during growth we have determined ^{59}Fe incorporation into red blood cells, serum IGF-I, and serum erythropoietin levels in normal growing rats.

The results obtained are supportive to the concept that erythropoiesis during growth is governed by IGF-I.

Materials and Methods

Animals

Sprague-Dawley rats (Ivanovas, Kissleg, FRG) were bred and grown in the local animal house. The animals had free access to standard chow (Altromin®) and water. Weights of animals were controlled daily at noon.

^{59}Fe-incorporation into red blood cells

Rates of erythropoiesis were determined by measuring iron incorporation into newly formed red blood cells. Rats were injected ip with 2μCi/100 g ^{59}Fe-chloride (specific activity 10μCi/μg); 48 h after the injection the rats were anesthesized with ether and bled by heart puncture. The 48-h interval was chosen to avoid interference with age-dependency of iron kinetics (10). Blood was collected in non-heparinized plastic tubes. Serum was stored at −20°C. The fractional ^{59}Fe incorporation into red blood cells (RBC) was calculated according to the following generally used formula: ^{59}Fe incorporation (%) = (radioactivity per ml of blood) × blood volume (ml) × 100/(total radioactivity injected). Blood volume was calculated according to published data (1). Total ^{59}Fe incorporation into RBC was calculated as (total radioactivity injected) × ^{59}Fe incorporation (%)/100.

Determination of serum erythropoietin concentrations

Serumerythropoietin levels were determined by radioimmunoassay as described previously (11). Erythropoietin-enriched rat serum was used as standard which had been calibrated in the exhypoxic mouse assay for erythropoietin (11). Each sample was measured in duplicate.

Determination of serum IGF-I concentrations

Serum IGF I was determined by radioimmunoassay (12) using rabbit antiserum batch 6656/251074 (gift from late Dr Reber, Hoffmann-La Roche, Basel, CH) against human IGF-I (final dilution 1:20 000). IGF-I was separated from IGF carrier proteins by gel permeation on a Sephadex G-50 column in 1 mol/l acetic acid. Separated IGF was lyophilized and stored at −20°C. After reconstitution with 1 ml PBS, pH 7.4, containing 0.1% (w/v) human serum albumin, all samples were assayed at 3 different dilutions.

Results

The development of body mass of Sprague-Dawley rats with aging is characterized by a sigmoidal curve (Fig. 1). A period of accelerated growth occurs between the 30th and 80th day after birth in both sexes. In this period, body weight gain ranges between 4.4 and 7.4 g/day in male and between 3.4 and 4.7 g/day in female rats. The age dependency of body weight gains for both sexes is shown in Fig. 1 (insert).

As indices for the activity of erythropoiesis both fractional and total ^{59}Fe incorporation into newly formed red blood cells were determined. Fractional ^{59}Fe incorporation was considered to reflect the specific activity of erythropoietic tissues (related to constant body mass), whereas total ^{59}Fe in-

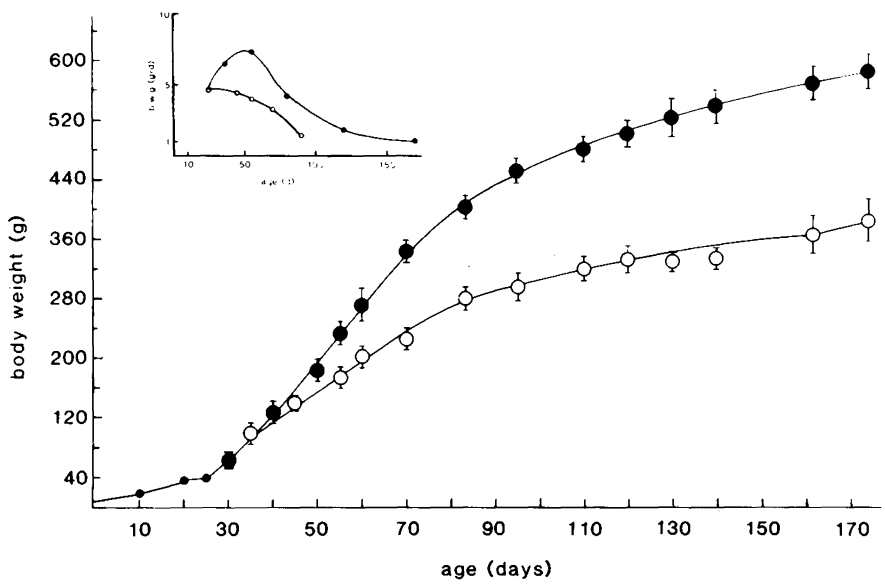

Fig. 1.

Body weight of male and female Sprague-Dawley rats as a function of age. Weights were determined daily. The values are shown for different ages as mean ± SEM (N = 20 to 30 for both sexes). Lines between the means were drawn by inspection (•) male, (○) female.

Insert: body weight gain (b.w.g.) at different ages for male (•) and female (○) rats.

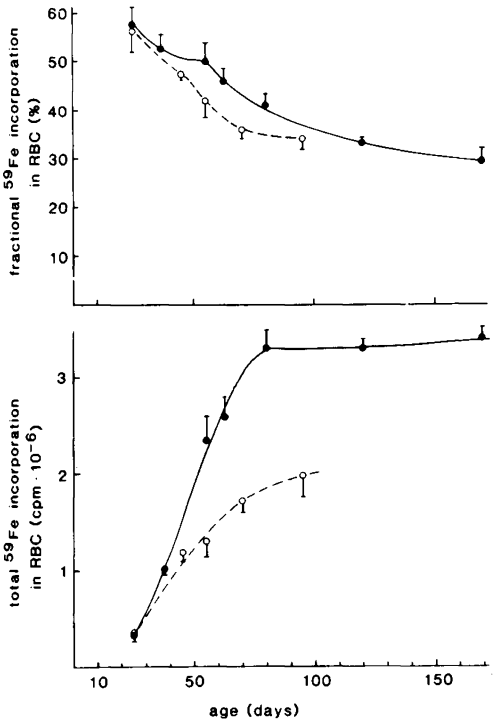

Fig. 2.
Fractional (upper panel) and total (lower panel) ^{59}Fe incorporation into newly formed red blood cells (RBC) in male (•) and female (○) rats at different ages. Data are mean ± SEM (N = 9 to 12 for both sexes).

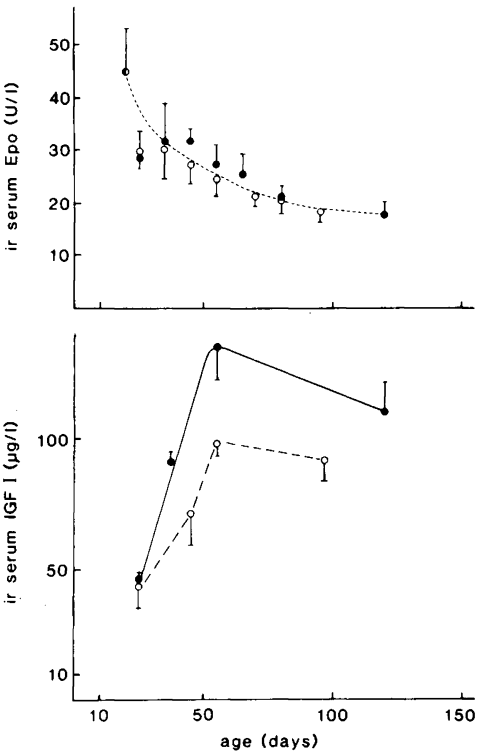

Fig. 3.
Immunoreactive serum erythropoietin (Epo) (upper panel) and serum IGF-I concentrations of male (•) and female (○) rats at different ages. Data are mean ± SEM (N = 7 to 9 for both sexes).

corporation reflects the absolute amount of red cells formed. The fractional incorporation rate of ^{59}Fe into red blood cells had a value of around 57% for both sexes at day 25 after birth (Fig. 2 upper panel) and declined to a value of around 30% with increasing age. ^{59}Fe incorporation rates in male animals decreased at a slower rate than those of female rats. Fractional ^{59}Fe incorporation rates and body weight gain were linearily correlated in both sexes (^{59}Fe incorporation (%) = 28.1 + 3.2 × body weight gain (g/day); r = 0.95). The total incorporation of ^{59}Fe into red blood cells increased almost linearily between day 30 and 70 (Fig. 3 lower panel) and approached a plateau with increasing age. The plateau in male rats was about 70% higher than that reached in female animals.

Serum-erythropoietin concentrations in both sexes decreased with increasing age in the time interval investigated (day 25-120) (Fig. 3, upper panel). Serum IGF-I concentrations on the other hand increased almost linearily during the phase of growth acceleration (Fig. 3, lower panel). Starting from the same level at day 25, IGF-I increased to around 135 µg/l in male and to around 95 µg/l in female rats. After the phase of accelerated growth, IGF-I levels tended to fall slightly in both sexes. In Fig. 4 the correlation between individual serum IGF-I levels and total rates of iron incorporation during the period of accelerated growth is shown. Both parameters are linearily correlated.

Discussion

In this study we have investigated the correlation between the activity of erythropoiesis, serum erythropoiesis, and serum IGF-I levels in growing rats. The results show that fractional ^{59}Fe incorporation, indicating specific erythropoietic activity, declines steadily from the neonatal period and onwards

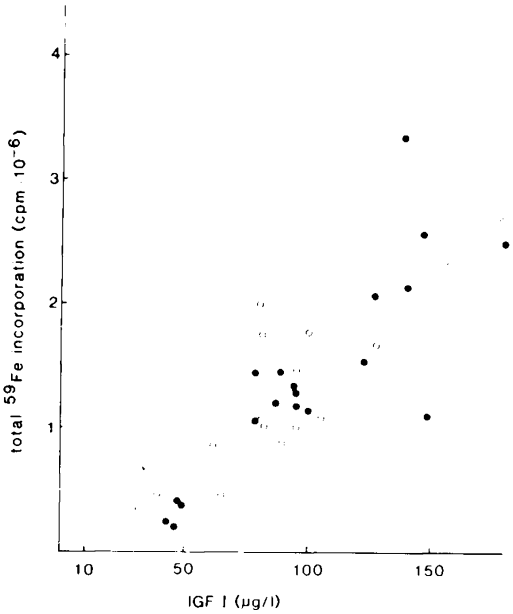

Fig. 4.
Relationship of individual values for total incorporation of ^{59}Fe into newly formed red blood cells to serum IGF-I levels in rats during accelerated growth. Linear regression as indicated by the dashed line: total ^{59}Fe incorporation (cpm 10^{-6}) = $-0.266 + 0.0166 \times$ [IGF-I] (µg/l); $r = 0.81$, $p<0.0001$. (•) male, (○) female animals.

(Fig. 2). This fall of iron incorporation rates is attenuated during the phase of accelerated growth, which occurs between 30 and 80 days after birth (Fig. 1). After the neonatal period, fractional ^{59}Fe incorporation rates were linearily correlated to the body weight gains. This observation supports the concept that fractional ^{59}Fe incorporation rates reflect the sum of red cell formation necessary to compensate daily red cell degradation and to refill the daily expanding circulatory system. Extrapolation of the linear regression curve yielded a fractional ^{59}Fe incorporation of 28% for non-growing animals. This value is similar to the ^{59}Fe incorporation rates of growth-arrested, hypophysectomized rats (9) determined under comparable experimental conditions.

Total red cell formation is reflected by the total incorporation of iron (Fig. 2, lower panel). The curve obtained fits well with direct calculations of daily red cell formation in rats during growth (1). Red cell formation increased by a factor of around 10 and 6 in male and female rats, respectively, during the period of accelerated growth. This increase in red cell formation was associated with a fall of serum erythropoietin levels (Fig. 3, upper panel). The observed decrease of serum erythropoietin with age is in accordance with observations by Clemons et al. (13) and Bozzini et al. (14), who also found a difference in serum erythropoietin between neonatal and adult rats. Most likely this decrease of erythropoietin formation is caused by recovery from the anemia which develops during the neonatal period, and which is completely compensated 70 days after birth in rats (1).

Considering the inverse relationship of serum erythropoietin to total red cell formation during accelerated growth, it seems unlikely that erythropoietin only governs the expansion of red cell volume in this period. The conclusion is in accordance with previous observations (15, 16) that erythropoiesis during rapid growth cannot be blunted by plethora, a condition which is known to suppress erythropoietin formation in the adult (17). Recently it has been shown that plethora in fact blunts erythropoietin formation but not the erythropoietic activity present in the serum of mice during rapid growth (18).

The temporal relation between IGF-I levels and body weight gain as observed in this study is in accordance with the concept that IGF-I governs the increase in body and organ mass during accelerated growth (3). Our findings show that red cell formation and serum IGF-I concentrations are also directly correlated during accelerated growth (Fig. 4). It is tempting to speculate therefore that IGF-I causes proliferation and expansion of hemopoietic tissues in proportion to the increase of body mass. Although our findings cannot prove a causality, they could provide an essential link in the understanding of a possible role of IGF-I in the regulation of erythropoiesis during growth. Whether this effect is mediated by systemic IGF-I or by locally released IGF-I (19) cannot be answered yet.

The present results show that red cell formation and body weight gain are linearily correlated, and that the individual rates of red cell formation are directly correlated with serum IGF-I levels. Recently it was demonstrated that infusion of IGF-I into growth-arrested hypophysectomized rats causes a proportional increase in growth and erythropoiesis (9). IGF-I, moreover, has been shown to enhance the proliferation of erythroid precursors

in vivo (4) and in vitro (5-8). In addition, the existence of IGF-I receptors on erythroid precusors (20) as well as the existence of IGF-I in the direct environment of erythroid precursors have been demonstrated in vivo (21). The sum of these observations supports the idea that IGF-I governs erythropoiesis during the period of accelerated growth and thus manages a proportional increase in body mass and oxygen transport capacity.

Such a role of IGF-I would not contest an essential involvement of erythropoietin in the maintenance of erythropoiesis in growing animals as suggested by a recent study (14). In fact, there is some evidence that IGF-I requires the presence of erythropoietin for its mitogenic effect on erythroid precusors from adult mammals (6-8). A modulatory role of IGF-I on erythropoiesis maintained by erythropoietin would also be supported by recent findings that serum erythropoietin levels do not change during puberty in humans (22, 23), a situation in which IGF-I levels rise and red cell masses expand.

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