Role of the Hypophysis in Erythropoietin Production During Hypoxia

By C. Peschle, I. A. Rappaport, M. C. Magli, G. Marone, F. Lettieri, C. Cillo, and A. S. Gordon

Hypophysectomized or sham-operated male rats were exposed to hypoxia (0.42-0.40 or 0.37-0.35 atm for 6, 12, or 24 hr) applied 2 wk to 7 mo after surgery. Erythropoietin (Ep) levels in rat serum were evaluated on the basis of the exhypoxic polycythemic mouse assay. Ep activity evoked by hypoxia was significantly lower in hypophysectomized rats than in sham-operated controls. Progressive increase of the Ep response to hypoxia correlated with extension of the time interval between hypophysectomy and hypoxia from 2 wk to 2-4 mo apparently mediated by the simultaneous inverse decline of red cell mass (RCM) values, i.e., of the "relative plethora" induced by a low O2 demand associated with relatively high RCM values. However, after 3-7 mo hypoxic Ep activity was still lower than in sham-operated controls. In these ablated animals the relative plethora became negligible or absent; accordingly, the Ep response apparently had reached plateau levels. These studies indicate that hypophysis (hypophyseal and target hormones, with the exception of estrogens) modulates Ep production under hypoxic conditions, possibly via a permissive enhancement of renal Ep activity.

EXTENSIVE EVIDENCE indicates that under physiologic conditions the endocrine system plays a major role in the regulation of erythropoiesis. Soon after removal of the pituitary, the metabolic rate declines rapidly and then levels off. This leads to a parallel decrease of erythropoietic activity, causing a slower, gradual reduction of red cell mass (RCM) values, which tapers off approximately 3-4 mo after hypophysectomy. Thus the initial, rapid decline of O2 consumption is thereafter compensated by a slower, progressive drop of O2 transport. Since it is conceded that erythropoietin (Ep) is the major regulator of physiologic red cell production, hypophysectomy apparently causes a sequential decline of O2 demand, Ep activity, erythropoietic rate, and RCM values.

The hypophyseal influence on normal erythropoiesis is clearly mediated by pituitary and target hormones. In hypophysectomized rats, regression of anemia is observed after combined treatment with GH, ACTH, corticosteroids, thyroid hormones, and testosterone. These agents apparently operate, at least partially, via elevation of Ep activity. However, androgens and corticosteroid derivatives may also exert a direct stimulatory effect on erythropoiesis. Of further interest is that in contrast with other target hormones estrogens in-
duce an inhibitory influence on red cell production; thus administration of physiologic amounts of estradiol in mice or rats causes a decline of either the number of erythroid colony-forming units in marrow or the level of hypoxic Ep activity in serum, respectively.

It is generally accepted that under hypoxic conditions hypophysis is not essential for Ep production. The present studies indicate that this concept should be partially reconsidered. In this regard, hypophysectomized rats showed a marked reduction of the Ep response to hypoxia applied at different time intervals (2 wk to 7 mo) after ablation of the pituitary. Thus it is postulated that during hypoxia pituitary and target hormones (except for estrogens) play a significant role in enhancing Ep production.

MATERIALS AND METHODS

Sprague-Dawley male rats weighing 90–150 g at the time of hypophysectomy were used. The animals, made available within 1 wk after the operation, were maintained on a normal diet of laboratory pellets and tap water ad libitum supplemented with 3% sucrose.

Erythropoietic activity in serum of hypophysectomized rats exposed to a bout of hypoxia (0.42–0.40 or 0.35–0.37 atm for 6–24 hr). In series 1 rats weighing 140–150 g at the time of hypophysectomy were exposed to hypoxia (either 0.42 or 0.35 0.37 atm of air/6 hr) at 2, 4, 6, 8, or 12 wk after the operation. Sham-operated controls of the same age were included only in experiments with milder hypoxia (0.42 atm). A minimum of 3 or 4 rats/group was used. Blood was collected by cardiac puncture under light ether anesthesia immediately after the end of the hypoxic period. Blood was pooled and centrifuged at 2000 rpm for 20 min. The serum was then collected and stored at −20°C. Pooled sera from all rats in each group were then assayed.

In some of those experiments, pooled sera from sham or hypophysectomized rats were incubated with either anti-Ep or normal rabbit serum (0.1 ml rabbit serum per 1 ml of rat serum) in a water bath incubator shaken for 60 min at 37°C. Thereafter, goat anti-rabbit gamma globulin (GARGG) (Antibodies Inc., Davis, Calif.) was incubated with the rat serum plus anti-Ep serum mixtures for an additional 15 min under the above conditions. An equivalent volume of saline was added in control vessels. The appropriate amount of GARGG was previously ascertained by testing against known quantities of anti-Ep. The precipitate resulting after the incubation with GARGG was discarded by centrifugation and the supernatant fluid was tested for Ep activity. One ml of this anti-Ep serum, obtained by a modification of the method by Schooley and Garcia, neutralized up to at least 125 or 12.5 IU of human or rat Ep, respectively.

In series 2 rats weighing 90–100 g at the time of hypophysectomy were exposed to hypoxia (0.40 or 0.35 atm/6 hr) at 3.5, 4, 4.5, 5, or 7 mo after the operation. Sham-operated controls of the same age were included in all experiments. A minimum of 3 or 4 rats/group was used. Bleeding, collection, storage, and assay of serum were performed as indicated above.

In series 3 rats weighing 90–100 g at the time of hypophysectomy were exposed to severe hypoxia (0.37 atm) for 6, 12, or 24 hr at 4 or 12 wk after the operation. Sham-operated controls were included in both experiments. Each group comprised a minimum of 3 or 4 rats. Bleeding, collection, storage, and assay of serum were performed as indicated above.

Assay of erythropoietic activity in hypoxic rat serum in exhypoxic polycythemic mice. CF₁ female mice weighing 20–25 g, maintained on a diet of standard laboratory pellets and tap water ad libitum, were used to assay the erythropoietic activity in all series of experiments.

In the first series, the mice were exposed to intermittent hypoxia (0.42 atm air/18 hr per day to a total of approximately 220 hr). Test sera or standard Ep* were injected intraperitoneally on days 6 and 7 after hypoxia. On day 8 the animals received intraperitoneally 0.5 μCi ⁵⁹Fe-citrate. In the second and third series of experiments the mice exposed to an equivalent hypoxic stimulus up to a total of 220 hr, were given test materials and standard Ep subcutaneously on days 3 and

*Sheep plasma Ep step I (Connaught Medical Research Laboratory, Toronto), standardized against International Reference Preparation (IRP) I or II (Division of Biological Standards, National Institute for Medical Research, London).

**Sheep plasma Ep Step III (Connaught) standardized against IRP II.
Fig. 1. Total RBC volume (means ± SEM) in rats at various times after either hypophysectomy or sham operation, as evaluated on the basis of the 59Fe-targeted RBC dilution technique. Minimum of three animals/group.

Fig. 2. Percentage RBC-59Fe incorporation values (means ± SEM) in assay mice treated on days 6 and 7 after hypoxia with serum from (A) sham-operated (---) or hypophysectomized (----) rats subjected to relatively mild hypoxia (0.42 atm/6 hr) or (B) hypophysectomized animals (---) exposed to more severe hypoxic stimulus (0.35–0.37 atm/6 hr). Hypoxia applied 2–12 wk after operation. Values derive from single assay of pooled sera of all rats in each group (minimum of 3 or 4 rats and 5 or 6 mice/group). Also indicated, radioiron uptake values in assay mice injected with standard Ep (0.05, 0.20, or 0.60 IU), as well as volume of donor serum/assay animal. Unoperated rats of same age were exposed to mild hypoxia simultaneously with the 2- and 8-wk groups; erythropoietic activity in serum was, respectively, 12.61 ± 0.54 and 11.20 ± 0.93 (mean percentage RBC-59Fe incorporation values ± SEM in assay mice, 0.5 ml/mouse).

RESULTS

RCM values in sham-operated or hypophysectomized rats. After hypophysectomy, RCM values initially showed a progressive decline that then slowed down and finally leveled off, starting from approximately 12 wk after the ablation (Fig. 1). It was further indicated that RCM values were then virtually unmodified up to 6 mo after sham operation.

Erythropoietic activity in serum of sham-operated versus hypophysectomized rats exposed to hypoxia. In preliminary studies the sera of rats subjected to hypoxia (0.35–0.37 atm/6 hr) applied at 8 or 16 wk after either hypophysec-
tomy or sham operation were incubated with anti-Ep and GARGG, causing complete neutralization of the erythropoietic activity in all sera: standards—saline, 0.42 ± 0.03 (mean 48-hr percentage RBC-59Fe incorporation values ± SEM in assay mice injected with test materials on days 6 and 7 after hypoxia); 0.05 IU Ep, 1.24 ± 0.37; 0.20 IU Ep, 6.28 ± 0.54; test materials—8- and 16-wk sham serum (0.1 ml/mouse), 5.71 ± 1.09 and 6.95 ± 0.88, respectively; these sera plus anti-Ep, 0.55 ± 0.07 and 0.62 ± 0.12. It would thus appear that in all experiments presented here the erythropoietic activity in serum of either sham-operated or hypophysectomized rats was totally referable to Ep. As indicated in Fig. 2, serum Ep levels in 2-12-wk hypophysectomized rats exposed to a 6-hr bout of hypoxia at either 0.35-0.37 or 0.40 atm were markedly lower than in sham-operated controls subjected to the hypoxic stimulus corresponding to 0.42 atm. In this series of experiments, limitations in the number of rats made available did not permit the inclusion of sham-operated controls in the experiments at 0.35-0.37 atm. However, as shown in Fig. 3, serum Ep levels were more elevated in sham-operated rats subjected to 0.35 than to 0.40 atm. Furthermore, additional studies indicated that Ep levels in rats subjected to a 6-hr bout of 0.35-0.37 atm hypoxia at 2, 6, or 12 wk after sham operation were respectively 2.50, 2.40, and 2.20 IU/ml serum. Thus it may be concluded that Ep activity in 2-12-wk hypophysectomized rats subjected to either 0.42- or 0.35-0.37 atm hypoxia was significantly reduced compared to that in sham-operated controls.

Serum Ep levels were further evaluated in rats subjected to severe or mild hypoxia (0.35 or 0.40 atm/6 hr) or 0.37 or 0.40 atm/6 hr) 3.5, 4, 4.5, 5, or 7 mo after hypophysectomy or sham operation (Figs. 3A* and 3B†). It is apparent that in all groups the hypoxia-induced Ep activity was lower in hypophysectomized rats than in sham-operated animals.

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*Corresponding mean ± SEM values of RBC-59Fe incorporation in assay mice (sham, S: hypophysectomy, H): S + 3.5 mo (0.2 ml serum/mouse), 24.84 ± 1.76; H + 3.5 mo (0.6), 18.98 ± 2.18; (standards: saline, not available; 0.05 IU Ep, 9.70 ± 2.08; 0.20 IU, 20.60 ± 1.13; 0.80 IU, 40.10 ± 1.54; H + 4 mo (0.3), 9.69 ± 1.10; (standards: 2.82 ± 0.44; 6.77 ± 0.63; 14.48 ± 1.07; 30.43 ± 1.77). S + 5 mo (0.2), 20.61 ± 1.89; H + 5 mo (0.5), 20.25 ± 3.02; (standards: 1.60 ± 0.26; 5.02 ± 0.61; 19.39 ± 1.81; 39.57 ± 0.97). S + 7 mo (0.2), 16.94 ± 0.85; H + 7 mo (0.6), 15.89 ± 1.72; (standards: 2.04 ± 0.39; 5.91 ± 0.41; 16.20 ± 1.07; not available). Unoperated rats of the same age were exposed to hypoxia simultaneously with the 3.5- and 5-mo groups: the serum erythropoietic activity, assayed simultaneously with that of the corresponding operated groups, was 27.02 ± 1.64 (0.2 ml serum/mouse) and 20.43 ± 1.51 (0.2), respectively.

†Corresponding mean ± SEM values of RBC-59Fe incorporation in assay mice (sham, S: hypophysectomy, H): S + 3.5 mo (0.1 ml serum/mouse), 22.48 ± 0.74; H + 3.5 mo (0.4), 16.16 ± 2.01; (standards: see values for corresponding age group in the footnote above). S + 4 mo (0.3), 31.78 ± 3.53; H + 4 mo (0.5), 15.29 ± 1.79; (standards: see footnote above). S + 4.5 mo (0.2), 20.26 ± 1.52; H + 4.5 mo (0.3), 3.57 ± 0.43; (standards: saline, 1.97 ± 0.09; 0.05 IU Ep, 5.36 ± 0.46; 0.20 IU, 12.94 ± 2.04; 0.80 IU, 23.32 ± 2.36). S + 5 mo (0.2), 29.89 ± 1.12; H + 5 mo (0.4), 24.16 ± 1.29; (standards: see footnote above). S + 7 mo (0.1), 15.10 ± 0.92; H + 7 mo (0.5), 16.03 ± 1.80; (standards: see footnote above). Unoperated rats of the same age were exposed to hypoxia simultaneously with the 3.5- and 5-mo groups: the serum erythropoietic activity, assayed simultaneously with that of the corresponding operated groups, was 21.36 ± 1.65 (0.1 ml/mouse) and 30.04 ± 2.32 (0.2), respectively.
Fig. 3. Ep levels (IU/ml serum) in sham (---) or hypophysectomized (-----) rats exposed to hypoxia (0.40 or 0.35 atm/6 hr) at different time intervals (3.5-7 mo) after the operation. Ep levels evaluated on the basis of a single assay of pooled sera of all rats in each group (minimum of three or four rats and five or six mice/group). *, p < 0.01 when comparing corresponding values of radioiron uptake, hypophysectomized versus sham.

Table 1. Ep Levels in Sera of Rats Subjected to Severe Hypoxia (0.37 atm) for 6, 12, or 24 hr 4 or 12 wk After Hypophysectomy or Sham Operation; Assay in Exhypoxic Polycythemic Mice

<table>
<thead>
<tr>
<th>Treatment of Donors</th>
<th>Volume of Donor Serum (ml/mouse)</th>
<th>Percentage RBC-^{59}Fe Incorporation Values in Assay Mice (m ± SEM)</th>
<th>Ep Levels in Donor Serum (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S + 4 wk + 6-hr hypoxia</td>
<td>0.10</td>
<td>13.07 ± 0.83</td>
<td>0.80</td>
</tr>
<tr>
<td>H + 4 wk + 6-hr hypoxia</td>
<td>0.40</td>
<td>3.47 ± 0.38</td>
<td>0.08</td>
</tr>
<tr>
<td>S + 4 wk + 12-hr hypoxia</td>
<td>0.20</td>
<td>36.45 ± 2.67</td>
<td>3.75</td>
</tr>
<tr>
<td>H + 4 wk + 12-hr hypoxia</td>
<td>0.40</td>
<td>16.00 ± 1.59</td>
<td>0.25</td>
</tr>
<tr>
<td>S + 4 wk + 24-hr hypoxia</td>
<td>0.20</td>
<td>25.75 ± 2.72</td>
<td>1.40</td>
</tr>
<tr>
<td>H + 4 wk + 24-hr hypoxia</td>
<td>0.40</td>
<td>8.69 ± 1.79</td>
<td>0.13</td>
</tr>
<tr>
<td>S + 12 wk + 6-hr hypoxia</td>
<td>0.20</td>
<td>20.01 ± 3.35</td>
<td>1.10</td>
</tr>
<tr>
<td>H + 12 wk + 6-hr hypoxia</td>
<td>0.40</td>
<td>4.53 ± 0.64</td>
<td>0.14</td>
</tr>
<tr>
<td>S + 12 wk + 12-hr hypoxia</td>
<td>0.20</td>
<td>28.28 ± 2.64</td>
<td>2.05</td>
</tr>
<tr>
<td>H + 12 wk + 12-hr hypoxia</td>
<td>0.40</td>
<td>11.85 ± 1.25</td>
<td>0.26</td>
</tr>
<tr>
<td>S + 12 wk + 24-hr hypoxia</td>
<td>0.20</td>
<td>27.40 ± 3.13</td>
<td>1.80</td>
</tr>
<tr>
<td>H + 12 wk + 24-hr hypoxia</td>
<td>0.40</td>
<td>14.00 ± 1.83</td>
<td>0.39</td>
</tr>
</tbody>
</table>

S, sham operation; H, hypophysectomy. Minimum of three donor rats and six mice per group. A single assay of pooled sera of all rats in each group was performed. Donor serum was injected on days 3 and 4 after hypoxia. For further details see Materials and Methods.
However, these studies did not include evaluation of the Ep response at sequential time intervals after the ablation, and more particularly in the later period, characterized by steady-state RCM values. Thus these observations did not consider the crucial role played in the phenomenon by the “relative plethora” observed in the early phase after hypophysectomy.

Table 2. Standard Ep (0.05, 0.2, 0.8 IU) 
(Mean Percentage RBC-59Fe Incorporation ± SEM in Assay Mice)

<table>
<thead>
<tr>
<th></th>
<th>4 wk</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>3.25 ± 0.74</td>
<td>1.55 ± 0.22</td>
</tr>
<tr>
<td>0.05 IU</td>
<td>8.64 ± 1.70</td>
<td>3.78 ± 0.68</td>
</tr>
<tr>
<td>0.20 IU</td>
<td>22.06 ± 0.68</td>
<td>19.00 ± 2.72</td>
</tr>
<tr>
<td>0.80 IU</td>
<td>38.41 ± 2.68</td>
<td>37.22 ± 1.87</td>
</tr>
</tbody>
</table>

Other details as in Table 1.

It is of interest that Ep levels in sera of hypophysectomized rats exposed to a 6-, 12-, or 24-hr period of severe hypoxia (0.37 atm) starting 1 or 3 mo after surgery were markedly lower than in corresponding sham groups (Table 1). Standard Ep incorporation values are shown in Table 2.

DISCUSSION

The present results indicate that the Ep activity evoked by hypoxia is significantly lower in hypophysectomized than sham-operated rats. This phenomenon was observed during both the “early” and the “late” phases after pituitary ablation, defined here, respectively, as the first 9–12 wk after the operation and the later period.

In regard to the early phase, Peschle et al.24 and then Schooley and Mahlmann25 preliminarily suggested that hypoxic Ep production is lower in hypophysectomized than in sham animals. In the present studies (Fig. 2) the Ep response in recently hypophysectomized rats was progressively increased by extension up to 9–12 wk between the operation and the hypoxic stimulus. It must be also emphasized, however, that Ep activity was always markedly lower in the hypophysectomized than the sham groups. As mentioned above, ablation of the hypophysis induces initially a relative plethora, i.e., normal RCM values with low O2 demand. Thereafter, RCM values gradually decline and taper off 3–4 mo after hypophysectomy; at this time the drop of O2 transport apparently corresponds to and compensates for the earlier decrease of O2 demand. Thus in the early phase after hypophysectomy the gradual increase of hypoxic Ep production is apparently mediated by the inverse decline of the O2 supply (i.e., of RCM values); this presumably renders the Ep-producing tissues more sensitive to the hypoxic stimulus. Accordingly, no significant difference in the P02O2 values during hypoxia was monitored between hypophysectomized and sham-operated rats.25

In regard to the late phase (starting 3–4 mo after operation), a diminished Ep response to hypoxia was observed in the hypophysectomized versus the corresponding sham animals (Fig. 3). As indicated above, these ablated rats were in a steady-state condition, i.e., the relative plethora was negligible or absent in that the decreased O2 demand was compensated by a corresponding drop of RCM values and hence O2 supply; accordingly, their Ep levels, although show-
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ing some fluctuations, apparently reached plateau levels. On the other hand, since their Ep response was still significantly dampened when compared with sham levels, it is apparent that pituitary and/or target hormones play a permissive role in enhancing hypoxic Ep production. In line with this concept, evidence has been presented indicating that several of these hormones potentiate serum Ep under normal or hypoxic conditions.8 13

The mechanisms underlying the hypophyseal influence on hypoxic Ep levels are of considerable interest. Evidence has been presented indicating that the activity of the renal erythropoietic factor (REF, a factor generating Ep after incubation with normal serum) was significantly more elevated in rodents injected with testosterone or GH than in vehicle-treated controls.12 13 Preliminary studies from our laboratory further suggest that REF activity is barely detectable in the kidneys of hypoxia-exposed, hypophysectomized rats. Thus hypophyseal and/or target hormones apparently potentiate the renal mechanisms underlying hypoxic Ep production. In the early phase after hypophysectomy the lack of these hormones may inhibit Ep production by dampening the hypoxic stimulus via elevation of the O2 supply/demand ratio in the whole body and more particularly at the level of the Ep-generating mechanisms. In the late period after pituitary ablation, an elevation of this ratio might persist selectively in tissues involved in Ep production; however, further studies will be necessary to elucidate these aspects.

Two levels of hypoxia have been used here in view of previous studies by Feigin and Gordon26 indicating differences in the erythroid responses of hypophysectomized rats subjected to mild or more severe hypoxia. Accordingly, hypophysectomized animals of corresponding ages showed here a higher level of serum Ep when subjected to a severe versus a milder hypoxic stimulus.

In conclusion, these studies indicate that the hypophysis (i.e., hypophyseal and target hormones, with the exception of estrogens) modulates Ep production under hypoxic conditions, apparently via permissive potentiation of the renal Ep-generating mechanisms.

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