Effect of Isobaric Hyperoxemia on Erythropoietin Secretion in Hypertensive Patients

Michał Kokot, Franciszek Kokot, Edward Franek, Andrzej Więczer, Michał Nowicki, Jan Dulawa

Abstract We assessed the influence of hyperoxemia on erythropoietin secretion in patients with various etiological forms of arterial hypertension (essential, n=15; renoparenchymal, n=16; renovascular, n=15) and in 15 healthy subjects. On the first day of the study, blood was withdrawn at 1-hour intervals for the estimation of erythropoietin during a total of 6 hours and at 2-hour intervals for the assessment of P02. Three days later the same parameters were assessed again at identical time intervals, but the subjects were breathing pure oxygen during the first 2 hours. Breathing with pure oxygen resulted in a significant increase of blood P02 (184.85±2.47 versus 85.92±2.28 in essential, 185.21±5.52 versus 84.55±3.04 in renoparenchymal, and 181.7±3.14 versus 87.49±2.25 in renovascular hypertension groups and 189.84±5.2 versus 85.89±1.73 mm Hg in healthy subjects; P<.001 in all groups). Baseline plasma erythropoietin was not different among the groups (29.33±4.14 in essential, 24.56±3.09 in renoparenchymal, and 27.77±3.29 in renovascular hypertension groups and 24.23±2.70 mU/mL in the control group). The pattern of erythropoietin decline was different in the groups of hypertensive patients. In patients with essential hypertension, unlike in healthy subjects and patients with other etiological forms of arterial hypertension, only a very short-term suppression of erythropoietin levels was observed during hyperoxemia. No significant changes in blood pressure during breathing with pure oxygen were found in any of the studied groups. We conclude the following: (1) Isobaric hyperoxemia exerts a significant suppressive effect on plasma erythropoietin levels both in patients with different etiological forms of arterial hypertension and in healthy subjects. (2) Patients with essential hypertension are characterized by a quantitatively different response of plasma erythropoietin to isobaric hyperoxemia compared with healthy subjects and patients with renoparenchymal or renovascular hypertension. (3) Oxygen physically dissolved in blood plasma seems to be an important determinant of erythropoiesis independent of hemoglobin concentration and oxygen saturation. (Hypertension. 1994;24:486-490.)

Key Words • erythropoietin • hypertension, essential • erythrocytosis • oxygen

When recombinant human erythropoietin (EPO) was introduced in the treatment of anemia caused by renal failure, the most important side effect of the therapy was an increase of blood pressure or worsening of preexisting hypertension.1,2 The proposed mechanisms of hypertension during EPO treatment were increased blood viscosity, loss of hypoxic vasodilation, and inappropriately reduced cardiac output after correction of anemia.2,4 Furthermore, recent studies suggested that EPO could induce a direct or hormonally mediated vasoconstrictor effect.3,6 The main stimulus for EPO secretion seems to be a decrease of oxygen supply to the renal tissue.7 As shown by Kurtz et al,7 EPO secretion is determined by the ratio of oxygen supply to oxygen demand in the kidney. The parameter that adequately reflects this ratio is P02 in small postcapillary peritubular veins. This local P02 is detected by a putative oxygen sensor regulating EPO secretion.7,8 Limited information is currently available on the influence of hyperoxia on the regulation of EPO secretion.9,11 Changes in renal hemodynamics and tubular function are well described in hypertension.12 According to the generally accepted concept of the regulation of EPO production, both of these factors might influence EPO secretion. Limited available information on endogenous EPO in arterial hypertension did not provide convincing evidence that plasma EPO levels are abnormal in this setting.13-15 In these studies abnormal regulation of EPO production could not be excluded. Existence of a likely link between blood pressure and EPO secretion stems from epidemiological data showing higher hematocrit and hemoglobin values in patients with arterial hypertension, thus suggesting that the physiological feedback mechanism regulating erythropoiesis could be altered in these patients.16-18 The above-mentioned facts formed the background for the present study, which assessed the influence of isobaric hyperoxemia on EPO secretion in patients with different etiological forms of arterial hypertension.

Methods A total of 46 patients were studied—15 with essential hypertension (EH), 16 with renoparenchymal hypertension (RH), and 15 with renovascular hypertension (RVH) —as well as 15 healthy control subjects (Table 1). In all patients blood pressure was higher than 160/95 mm Hg when assessed in the supine position on at least two occasions. Patients with congestive heart failure, diabetes, nephrotic syndrome, liver disease, renal failure, or malignant hypertension were excluded. All subjects gave informed consent; the study protocol was approved by the Local Ethics Committee.

Essential hypertension was diagnosed after exclusion of secondary causes of hypertension. The RH group consisted of patients with chronic glomerulonephritis (n=7) or chronic pyelonephritis (n=9). The patients with glomerulonephritis had proteinuria greater than 0.5 g/24 h and/or erythrocytura...
and no changes in urography, and the diagnosis was confirmed by renal biopsy. Patients with pyelonephritis had urine specific gravity lower than 1.018 after 12-hour hydropenia, recurrent leukocyturia and bacteriuria, and morphological abnormalities of the kidney confirmed by urography. The diagnosis of renovascular hypertension was based on the following criteria: significant stenosis of the renal artery confirmed by renography, elevation of plasma renin activity in renal venous blood originating from the ischemic kidney, ratio of plasma renin activity in renal venous blood of the ischemic kidney to that of the normally perfused one greater than 1.5, and smaller size of the ischemic kidney by 3 cm in length and 1.5 cm in width. All medications were withdrawn on admission at least 10 days before the study. During the entire hospitalization period the subjects were on a standardized diet containing 120 mmol sodium per 24 hours. The total water supply ranged from 1.5 to 2.0 L/24 h.

In all studied subjects two tests were performed according to the following protocol. On the first day of the study at 7:30 AM venous blood samples were withdrawn from recumbent and air-breathing subjects for the estimation of erythrocyte count; hemoglobin concentration; plasma levels of creatinine, iron, and ferritin; and total iron binding capacity. Immediately after blood withdrawal all subjects remained supine for 6 hours. At 1-hour intervals blood was withdrawn for the estimation of EPO and at 2-hour intervals for the assessment of PO2. In addition, mean arterial blood pressure was measured at 1-hour intervals. Three days later all above-mentioned parameters were assessed again at identical time intervals. The only difference was the administration of pure oxygen for respiration during the first 2 hours. Oxygen was administered with a facial mask (oxygen flow, 6 L/min). EPO concentrations were measured by radioimmunoassay. EPO (from Boehringer Mannheim) was labeled with 125I using the chloramine method. Sera and standards were incubated with EPO antibodies (raised in rabbits; final dilution of EPO antibodies, 1:60 000) for 24 hours at 4°C. Then 125I-EPO was added and the incubation extended for a further 24 hours. Antibody-bound EPO was precipitated using anti-γ-globulin antibodies with polyethylene glycol. The interassay and intra-assay coefficients of variability were 15% and 9%, respectively, and the sensitivity was 1.25 mU per tube. Ferritin concentration was assessed radioimmunologically with a kit from Amersham. PO2 was assessed in arterialized capillary blood withdrawn from the finger by the micro-Astrup method using a Corning type 158 apparatus. Other parameters were measured by routine laboratory methods.19

Statistical analysis was performed with ANOVA and Student’s paired and unpaired t test, respectively. Regression lines were calculated using the least-squares method. All results are expressed as mean±SEM. A value of P<.05 was considered statistically significant.

Results

All examined groups did not differ significantly with regard to age, body weight, erythrocyte count, and plasma concentrations of hemoglobin, iron, and ferritin (Tables 1, 2, and 3). In RH patients the creatinine level was moderately and significantly higher than in control subjects (Table 1).

Mean arterial blood pressure was significantly higher in the EH, RH, and RVH groups than in control subjects (Table 2). Neither air (first test) nor oxygen breathing (second test) influenced mean arterial pressure (data not presented).

Compared with PO2 values obtained during the first day of the test (air breathing) (Table 4), pure oxygen breathing was followed by a significant and similar increase of PO2 in all examined groups (P<.001) (Table 5).

Basal (at hour zero) plasma levels of EPO at the first and second days of the study were of similar magnitude in all examined groups (Figs 1 and 2). After 4 to 5 hours of air breathing, a moderate increase of plasma EPO level was noticed in all groups, and this increase was significant in the EH and RH groups and in control subjects (P<.05).

As can be seen in Fig 2, pure oxygen breathing was followed by a significant decrease of plasma EPO level. In the RH group this decrease was present up to the fourth hour, in control subjects up to the fifth hour, and in the RVH group up to the sixth hour (P<.05). In the EH group a significant decrease of plasma EPO con-

### Table 1. Clinical Data of Investigated Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age, y</th>
<th>Body Weight, kg</th>
<th>Serum Creatinine, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH (n=15)</td>
<td>6M, 9F</td>
<td>38.07±3.15</td>
<td>70.27±2.69</td>
<td>90.79±4.54</td>
</tr>
<tr>
<td>RH (n=16)</td>
<td>9M, 7F</td>
<td>41.81±2.17</td>
<td>75.69±1.75</td>
<td>105.62±5.0*</td>
</tr>
<tr>
<td>RVH (n=15)</td>
<td>10M, 5F</td>
<td>47.09±3.24</td>
<td>74.80±1.88</td>
<td>90.23±4.58</td>
</tr>
<tr>
<td>C (n=15)</td>
<td>9M, 6F</td>
<td>39.40±2.41</td>
<td>73.01±1.58</td>
<td>86.53±3.31</td>
</tr>
</tbody>
</table>

EH indicates patients with essential hypertension; RH, patients with renoparenchymal hypertension; RVH, patients with renovascular hypertension; and C, healthy control subjects. Values are mean±SEM.

*P<.05 compared with healthy subjects.

### Table 2. Hemoglobin Concentration, Erythrocyte Count, Hematocrit, and Mean Arterial Blood Pressure at the Beginning of the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Hemoglobin, mmol/L</th>
<th>Erythrocytes, 1012/L</th>
<th>Hematocrit</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>4.27±0.11</td>
<td>8.26±0.2</td>
<td>0.419±0.01</td>
<td>115.6±4.55*</td>
</tr>
<tr>
<td>RH</td>
<td>4.23±0.14</td>
<td>8.19±0.26</td>
<td>0.414±0.013</td>
<td>113.0±3.85*</td>
</tr>
<tr>
<td>RVH</td>
<td>4.05±0.13</td>
<td>7.82±0.26</td>
<td>0.397±0.013</td>
<td>119.4±5.20*</td>
</tr>
<tr>
<td>C</td>
<td>4.25±0.09</td>
<td>8.17±0.17</td>
<td>0.416±0.009</td>
<td>95.9±2.19</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; groups as defined in Table 1. Assessments were made on the first day at 7:30 AM. Values are mean±SEM.

*P<.05 compared with healthy subjects.
when respective plasma EPO levels assessed in the same time points in the individual groups on the first and second days of the study were compared, significantly lower EPO concentrations were found after oxygen breathing in the RH group at the second, third, and fourth hours; in the RVH group at the fourth and fifth hours; and in control subjects at the fourth, fifth, and sixth hours \( (P<.05 \) in all groups). In EH patients respective EPO levels obtained on days 1 and 2 were not statistically different.

No significant correlations were found between EPO levels and hemoglobin concentration and \( P_02 \), respectively.

### Discussion

The regulation of EPO secretion in subjects with or without anemia has been the subject of many investigations.\(^7,8,20-23\) From those studies it follows that the metabolic factor that determines the magnitude of EPO secretion is the ratio of oxygen supply to oxygen consumption in the kidney tissue.\(^24\) It is generally accepted that this ratio is best reflected by the \( P_02 \) in small postcapillary veins in the vicinity of the renal proximal tubules.\(^24\) Renal oxygen consumption in its turn is mainly determined by tubular sodium reabsorption.\(^25\) This venous \( P_02 \) is supposed to be an appropriate stimulus for the EPO secretion–regulating oxygen sensor, which is probably a hemoprotein.\(^7,8,24\)

Much less is known about the influence of elevated arterial \( P_02 \) (above the physiological range) on EPO secretion in subjects with a normal blood hemoglobin concentration.\(^9-12\) Under physiological conditions, arterial \( P_02 \) is maintained between 80 and 100 mm Hg, and in this range hemoglobin is nearly completely saturated. As for each 1 mm Hg of \( P_02 \), the amount of physically dissolved oxygen increases only by 0.031 mL; the amount of physically dissolved oxygen in 1 L of blood at a \( P_02 \) of 100 mm Hg is 3.1 mL. On the other hand, the amount of oxygen bound by hemoglobin (assuming a hemoglobin concentration of 9.3 mmol/L and complete oxygen saturation) amounts to 208.3 mL per 1 L of blood. Thus, the total oxygen content in 1 L of completely oxygen saturated blood is 211.4 mL. In our studies the peak \( P_02 \) after pure oxygen breathing was approximately 200 mm Hg, meaning that the total oxygen content in 1 L of blood increased by only 3.1 mL compared with that under air-breathing conditions at a \( P_02 \) of 100 mm Hg. This means an increase in the total oxygen content of only 1.5%. Despite such a small relative increase of oxygen content, we found a significant decrease of plasma EPO concentration in all examined groups. This fact is corroborated by the early study of Linman and Pierre,\(^26\) which provided evidence of a suppression of erythropoiesis in rats under hyperbaric conditions. In our study, time of onset and duration of EPO suppression were different in the groups. The peak decrease of EPO was approximately 25% in the EH group at 2 hours, 36% in the RH group at 3 hours, 32% in the RVH group at 4 hours, and 31% in control subjects at 3 hours compared with respective baseline values.

The question arises: How can the EPO-lowering effect of pure oxygen breathing be explained? As is well known, the serum concentration of a given hormone is the result of a dynamic balance between its secretion to and elimination from blood. Assuming a complete cessation of secretion of a hormone, the elimination rate can be presented by the following equation:

\[
N = N_0e^{-bt}
\]

where \( N_0 \) is the concentration of a hormone at time 0, \( b \) is the constant of elimination, \( N \) is the concentration of a hormone at time \( t \), and \( e \) is the base number of the natural logarithm.

From this equation it follows that the constant \( b \) describes the shape of the elimination curve and determines the half-life time \( (T_{\frac{1}{2}}) \) of a hormone, which is what follows from the following equation:

### Table 3. Iron Metabolism Parameters Assessed at the Beginning of the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Iron, ( \mu mol/L )</th>
<th>Serum Ferritin, ( \mu g/L )</th>
<th>TIBC, ( \mu mol/L )</th>
<th>Saturation Index (Fe/TIBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>18.42±1.42</td>
<td>132.31±31.28</td>
<td>70.09±4.86</td>
<td>0.257±0.024</td>
</tr>
<tr>
<td>RH</td>
<td>18.25±6.56</td>
<td>141.75±31.0</td>
<td>81.15±4.71</td>
<td>0.241±0.03</td>
</tr>
<tr>
<td>RVH</td>
<td>15.59±1.20</td>
<td>130.50±16.94</td>
<td>77.20±4.70</td>
<td>0.202±0.038</td>
</tr>
<tr>
<td>C</td>
<td>18.56±2.05</td>
<td>127.67±24.75</td>
<td>77.62±6.23</td>
<td>0.261±0.044</td>
</tr>
</tbody>
</table>

\( Fe \) indicates serum iron; \( TIBC \), total iron binding capacity; and groups as defined in Table 1. Assessments were made on the first day at 7:30 AM. Values are mean±SEM.

### Table 4. Partial Oxygen Pressure in Arterialized Capillary Blood on the First Day of the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>( P_02 ), ( mm \ Hg )</th>
<th>( P_02 ), ( mm \ Hg )</th>
<th>( P_02 ), ( mm \ Hg )</th>
<th>( P_02 ), ( mm \ Hg )</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>88.38±2.95</td>
<td>87.37±2.70</td>
<td>87.09±3.35</td>
<td>87.40±3.66</td>
</tr>
<tr>
<td>RH</td>
<td>84.39±1.89</td>
<td>82.57±2.65</td>
<td>81.55±1.49</td>
<td>87.68±4.65</td>
</tr>
<tr>
<td>RVH</td>
<td>84.08±2.47</td>
<td>81.0±2.78</td>
<td>85.68±2.59</td>
<td>89.25±3.05</td>
</tr>
<tr>
<td>C</td>
<td>90.60±2.91</td>
<td>86.23±3.45</td>
<td>86.70±4.43</td>
<td>89.95±2.84</td>
</tr>
</tbody>
</table>

Groups are as defined in Table 1. Shown are \( P_02 \) at the beginning (\( P_02 \)-0) and after 2 (\( P_02 \)-2), 4 (\( P_02 \)-4), and 6 hours (\( P_02 \)-6) of air breathing. Values are mean±SEM.
TABLE 5. Partial Oxygen Pressure in Arterialized Capillary Blood on the Second Day of the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Air Breathing PO₂-0, mm Hg</th>
<th>Pure O₂ Breathing PO₂-2, mm Hg</th>
<th>Air Breathing PO₂-4, mm Hg</th>
<th>Air Breathing PO₂-6, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>85.92±2.28</td>
<td>184.85±4.47*</td>
<td>86.46±2.6</td>
<td>91.62±2.02</td>
</tr>
<tr>
<td>RH</td>
<td>84.55±3.04</td>
<td>185.21±5.52*</td>
<td>85.39±3.17</td>
<td>90.94±2.82</td>
</tr>
<tr>
<td>RVH</td>
<td>87.49±2.25</td>
<td>181.70±3.14*</td>
<td>85.78±2.59</td>
<td>90.23±2.47</td>
</tr>
<tr>
<td>C</td>
<td>85.89±1.73</td>
<td>189.84±5.2*</td>
<td>89.37±2.17</td>
<td>93.80±1.62</td>
</tr>
</tbody>
</table>

Groups are as defined in Table 1. Shown are PO₂ before (PO₂-0) and after 2 hours (PO₂-2) of pure oxygen breathing and 2 (PO₂-4) and 4 hours (PO₂-6) after discontinued pure oxygen administration. Values are mean±SEM.

*P<.001 compared with PO₂-0, PO₂-4, and PO₂-6.

\[
\frac{\ln 2}{0.693} = \frac{1}{b} \Rightarrow b = \frac{1}{\frac{\ln 2}{0.693}}
\]

**T_{1/2}** defines the elimination time of half the amount of a given substance. The decrease of plasma concentration of a substance after time t (expressed as a percent, D%) is expressed by the equation

\[
D\% = 100\% - 100\% \times e^{-bt}
\]

From equation 3 one can calculate the constant b. The half-life time of EPO ranges from 5 to 13 hours. Accepting 5 hours as the shortest **T_{1/2}**, the calculated b value is 0.1386.

From equation 3 one can calculate the theoretical percent decrease of EPO level after time t, which is 24% after 2 hours, 34% after 3 hours, and 43% after 4 hours. In fact, the highest decrease of plasma EPO level in the EH group was 25% (at 2 hours), 36% in the RH group (at 3 hours), 32% in the RVH group (at 4 hours), and 31% in control subjects (at 3 hours). These data show that the theoretical values of EPO elimination were superimposable with those obtained in all groups (with the exception of the RVH group).

Because of this consistency between data obtained in our study and the theoretically calculated EPO elimination curve, it seems likely that a rise in PO₂ by approximately 100%, which caused only a slight increase in oxygen supply to the kidneys, had a distinct lowering effect on serum EPO concentration and could be followed by a complete cessation of EPO secretion. But one should also consider the other possibility that the observed decline of EPO might be caused by the simultaneous partial decrease of EPO secretion and increase of total EPO clearance. We did not study EPO elimination in our patients. We are not aware of any studies addressing the problem of changes in the metabolic clearance rate of EPO under normobaric hyperoxemia. As Pagel et al. showed that hyperoxemia did not change either renal hemodynamics or whole-kidney oxygen consumption, it seems unlikely that changes in these two factors are a dominant cause of decreased EPO secretion or increased renal EPO elimination during oxygen breathing.

Schurek et al. found that because of the existence of oxygen diffusion shunt, a substantial amount of oxygen shifts from the intrarenal arterial to venous vessels before the blood reaches the postcapillary peritubular veins. Thus it is doubtful that the 1.5% increase in oxygen blood content (as found in the present study) could affect PO₂ in the postcapillary peritubular veins. Therefore, the question arises whether high PO₂ might suppress EPO secretion in a way other than through intrarenal sensors. Despite some controversies, data suggest that the central nervous system may influence EPO secretion. It is also known that the nervous system has sensors for arterial PO₂ (carotid glomus). Recently, Pagel et al. reported on the presence of a
humoral factor stemming from the central nervous system that had a regulating effect on renal EPO secretion. Consequently, the possibility that high PO2 suppresses EPO secretion via the nervous system should be considered. Although the precise mechanism of the effect of pure oxygen breathing remains to be elucidated, it seems that physically dissolved arterial oxygen itself is an important factor influencing serum EPO level, besides hemoglobin concentration and oxygen saturation.

Baseline EPO levels found in our patients ranged from 23 to 30 mU/mL and were similar in magnitude to those reported by other authors31 and in our previous studies but were higher than those published by Mansion-Garcia et al. This discrepancy seems to be due to differences between the EPO antibodies these authors used in their radioimmunoassays.

From data obtained during air breathing, we can conclude that EPO secretion shows a circadian rhythm that was accentuated most in healthy subjects and RH patients. In healthy subjects the lowest EPO level was found at 8 to 9 AM and the highest at 8 to 9 PM. As shown in the present study, pure oxygen breathing induced a suppressive effect on plasma EPO levels in all examined groups, although the onset, magnitude, and duration of this effect were different in the individual groups. The lowest effect was noticed in EH patients. These results suggest that the pathophysiologic effect of hyperoxemia may influence the response of EPO secretion to hyperoxemia. The hyperoxemia-induced short-term decrease of EPO level did not affect blood pressure in our study.

Thus, despite evidence of a hypotensiveigenic effect of EPO, the role of EPO in the pathogenesis of arterial hypertension is unresolved.

We conclude the following: (1) Isobaric hyperoxia exerts a significant suppressive effect on plasma EPO levels in patients with different etiologic forms of arterial hypertension and in healthy subjects. (2) EH patients are characterized by a quantitatively different response of plasma EPO levels to isobaric hyperoxemia compared with healthy subjects and RH and RVH patients. (3) Oxygen physically dissolved in blood plasma seems to be an important determinant of EPO secretion independent of hemoglobin concentration and oxygen saturation.

References
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