Is the renal production of erythropoietin controlled by the brain stem?

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Submitted 26 April 2004; accepted in final form 20 February 2005

Von Wussow, Ursula, Janina Klaus, and Horst Pagel. Is the renal production of erythropoietin controlled by the brain stem? Am J Physiol Endocrinol Metab 289: E82–E86, 2005. First published February 22, 2005; doi:10.1152/ajpendo.00182.2004.—Although the structure and function of erythropoietin (Epo) are well documented, the mechanisms of the regulation of the renal synthesis of Epo are still poorly understood. Especially, the description of the localization and function of the O$_2$-sensitive sensor regulating the renal synthesis of Epo is insufficient. A body of evidence suggests that extrarenal O$_2$-sensitive sensors, localized particularly in the brain stem, play an important role in this connection. To support this concept, high cerebral pressure with consecutive hypoxia of the brain stem was generated by insufflation of synthetic cerebrospinal fluid into the catherized cisterna magna of rats. When the cerebral pressure of the rats was above the level of their mean arterial blood pressure or the high cerebral pressure persisted for a longer period (>10 min), the Epo plasma concentration increased significantly. Bilateral nephrectomy or hypophysectomy before initiation of high intracranial pressure abolished this effect. Systemic parameters (heart rate, blood pressure, $P_{A_O_2}$, $P_{A_CO_2}$, arterial pH, renal blood flow, glucose concentration in blood) were not affected. Other stressors, like restricting the mobility of the rats, had no effect on Epo production. Hence, the effect of high cerebral pressure on renal synthesis of Epo seems to be specific. Increasing cerebral hydrostatic pressure leads to increased renal synthesis of Epo. Obviously, during hypoxia, cerebral O$_2$-sensitive sensors release humoral factors, triggering the renal synthesis of Epo. The structure and function of these “Epo-releasing-factors” will have to be characterized in future experiments.

ERYTHROPOIETIN (Epo), a GLYCOPROTEIN HORMONE produced primarily by the adult kidney, is responsible for the regulation of the production rate of red blood cells. The structure and function of the molecule are well documented (for an overview, see Ref. 19). Binding to its membrane-standing receptor prevents apoptosis of the constitutively formed descendants of the stem cells in the bone marrow and leads to increased DNA and RNA synthesis, cell division, and synthesis of hemoglobin in the erythroid precursors.

About 20 years ago, the Epo gene was cloned. This was the starting point for large-scale production of the recombinant human Epo (rhEpo). Today, the application of rhEpo is the “gold standard” in the therapy of renal anemia (20).

On the other hand, the mechanisms regulating the renal synthesis of Epo are still poorly understood. In general, it is assumed that the oxygen-sensitive sensor triggering the synthesis of Epo is located in the renal cortex (1). This hypothesis is in agreement with the finding that reduction of the arterial oxygen partial pressure ($P_{A_O_2}$) leads to a small increase in Epo production in isolated perfused kidneys (27). It is supposed by studies in cell cultures that the oxygen sensor may be localized in the cells producing Epo (13).

However, in experimental (25) and clinical (14) studies, data were obtained indicating that additional extrarenal sensors are involved in the regulation of the synthesis of Epo.

A reduction in renal blood flow with a consecutive lowering of the oxygen supply to the kidney is not answered by adequate enhancement of the synthesis of Epo (3, 9, 23, 26). Shunt perfusions of venous blood from the right atrium of the heart to the renal artery leads to only a slight increase of plasma Epo levels (30). Patients with extreme renal artery stenosis show only occasionally and, if then, only minimal increases in plasma Epo levels (14). Polycythemia is a very rare consequence of renal artery stenosis.

These data reveal that extrarenal oxygen-sensitive sensors play an important role in the regulation of intrarenal Epo synthesis. Like the manner in which the synthesis of other hormones is regulated, the top authority in the regulation of the synthesis of Epo may be localized in the hypothalamic-hypophyseal system. It was shown that hypophysectomized animals are not able to respond to a hypoxic stimulus with increased Epo production. Electrical stimulation of the hypothalamic region leads to an elevated Epo concentration in blood (15).

The present investigation was done to develop an experimental model in which the brain of a rat was exposed to hypoxia while the rest of the organism was kept normoxic. This was achieved by inserting a catheter into the cisterna magna. Via this catheter, the hydrostatic pressure in the area of the brain stem was elevated above the level of the mean arterial blood pressure by insufflation of synthetic cerebrospinal fluid. This maneuver had the consequence that oxygen transport across the blood-brain barrier was substantially reduced or stopped. This led to a strong, transient hypoxia in the area of the brain stem. According to our hypothesis, cerebral oxygen sensors should respond with the release of humoral factor(s) triggering the Epo synthesis of the kidney.

EXPERIMENTAL PROCEDURES

Experimental animals. Normal and hypophysectomized adult male Sprague-Dawley rats were obtained from a local animal farm (Charles River, Kisslegg, Germany; 200–400 g body wt). To get bilaterally nephrectomized rats, their kidneys were exposed by flank incisions, and the renal pedicles were ligated. The animals had free access to food and water until the experiments.

General anesthesia was induced by intraperitoneal injection of 50 mg of pentobarbital sodium/kg body wt. Thereafter, the animals were placed in a dorsal position on a temperature-controlled operating table. The body temperature was kept constant at 37.5°C and monitored with a rectal probe. To ensure a constant oxygen supply, the animals underwent a tracheotomy and were ventilated with room air.

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or pure oxygen (Rodent Ventilator UB 7025; Ugo Basile, Comerio, Italy; tidal volume 2.5 ml, respiration rate 80 min⁻¹). The effectiveness of the ventilation was checked by blood gas analysis once an hour.

To be able to apply substances (intravenous bolus injection of pentobarbital sodium in the case of low narcosis, dopamine in the case of a drop in mean arterial blood pressure < 80 mmHg, substitution of volume by a solution containing hetastarch in 0.9% NaCl), one vena femoralis was catheterized. For the taking of blood samples (blood gas analyses, determinations of Epo concentrations), another catheter was placed into one arteria femoralis. Before insertion, the catheters (PE-50) were heparinized.

Thereafter, the rats were placed in a ventral position on a stereotactic table. The head was tilted ~45° and fixed in this position. After the membrana atlantooccipitalis posterior was laid open, the cisterna magna (i.e., cisterna cerebellomedullaris) was maximally exposed.

Heart rate was depicted with an oscilloscope. The mean arterial pressure was taken with a pressure transducer and recorded on a line recorder (Fig. 1, top).

**Experimental procedure.** To increase the intracranial pressure (ICP), a catheter was placed into the cisterna magna (length 3–4 mm, OD 0.96 mm, ID 0.58 mm; Fig. 1, bottom). The catheter was fixed with superglue and connected to a flask containing synthetic cerebrospinal fluid (Na⁺ 150 mM, K⁺ 3 mM, Ca²⁺ 1.4 mM, Mg²⁺ 0.8 mM, Cl⁻ 155 mM in phosphate buffer, pH 7.4). An increase in ICP was achieved by pressure-controlled insufflation of the synthetic cerebrospinal fluid into the cisterna magna.

The rats were divided into the following experimental groups: group 1 (respiration with air, n = 4 each): increased ICP to 60, 80, 100, or 120 mmHg for 10 min; group 2 (respiration with oxygen, n = 4 each): increased ICP to 100 mmHg for 5, 10, 15, or 30 min; group 3 (respiration with oxygen, n = 3): increased ICP to 100 mmHg for 10 min; group 4 (respiration with air, bilateral nephrectomy, n = 4): increased ICP to 100 mmHg for 10 min; and group 5 (respiration with air, hypophysectomy, n = 5): increased ICP to 100 mmHg for 10 min.

Controls were represented by two groups of animals; one group was ventilated with air (n = 5) and the other with oxygen (n = 4). Both control groups had no increased ICP.

To be able to measure renal blood flow before and during the induction of increased ICP, four additional anesthetized animals underwent a laparotomy along the linea alba. Thereafter, an ultrasonic flow probe was placed around a renal artery (T106; Transonic Systems, Ithaca, NY). The procedure to induce increased ICP was the same as described above.

Unspecific stress treatment consisted of restricting the mobility of five additional rats by placing them in Plexiglas restraints. The animals had no ability to move for 30 min.

**Taking of samples,** After a preperiod of 1 h, 0.5 ml of blood was taken from the catheter in the arteria femoralis once an hour (4 blood samples all together; Fig. 2). Two-tenths of a milliliter was used for blood gas analysis. After centrifugation, the plasma of the remaining samples all together; Fig. 2). Two-tenths of a milliliter was used for blood gas analysis. After centrifugation, the plasma of the remaining

**Analyses.** Plasma concentrations of Epo were determined by means of a commercially available ELISA (Medac, Hamburg, Germany) according to the manufacturer’s instructions. Instead of human Epo standards, rat standards were used, calibrated with the in vivo method after Ref. 4. Glucose plasma concentrations were likewise analyzed with a commercial assay (hexokinase method; Roche, Darmstadt, Germany).

**Statistics.** The results are given as means ± SE. To compare a control group with different treatment groups, Dunnett’s test was used. For comparison of different treatment groups among one another, the Tukey-Kramer test was carried out. The level of significance was set at P < 0.05.

**RESULTS**

**Epo after increase in ICP.** Figure 3 shows that the plasma Epo concentration increased after a transient increase in ICP in a pressure- and time-dependent manner, especially in those cases when ICP was elevated above the level of the mean...
arterial blood pressure (Fig. 3, top) or when the increase of ICP persisted longer than 10 min (Fig. 3, bottom).

Bilateral nephrectomy or hypophysectomy before the elevation of ICP abolished this effect (Fig. 4). This indicates that the ICP-induced increase of Epo production was accomplished primarily by the kidney. In addition, the hypophysis seemed to play an important role in this connection.

To exclude the possibility that the ventilation of the animals with air caused a mild systemic hypoxia, which might have initiated increased Epo production, a group of rats was ventilated with pure oxygen. In these experiments, the $\text{PaO}_2$ was $494 \pm 13 \text{ mmHg}$. However, as illustrated in Fig. 5, ventilation with oxygen had no influence on ICP-induced Epo production. Neither in the control groups nor in the treated groups was any statistically significant difference in the respective Epo plasma concentration measurable. On the other hand, comparison of the oxygen-ventilated control group with the respective treatment group yielded a significant increase of the Epo concentration in the plasma of the treated animals. The hyperoxia caused by the ventilation with pure oxygen could not prevent the ICP-induced increase in Epo production.

Restriction of the mobility of the rats by placing them in Plexiglas restraints had no influence on Epo production (Epo under control conditions: $5.6 \pm 2.1 \text{ mU/ml}$; Epo under restriction of mobility: $5.9 \pm 1.7 \text{ mU/ml}$; $n = 5$ each).

Systemic parameters. The induction of increased ICP had no influence on systemic parameters. The heart rate did not change during the experiments; in addition, there were no differences between the experimental groups (overall mean: $401 \pm 4 \text{ min}^{-1}$).

The mean arterial blood pressure amounted to $116 \pm 3 \text{ mmHg}$ at the beginning of the experiments (no differences between groups). Immediately after induction of increased ICP, mean arterial blood pressure increased statistically significantly to $126 \pm 1 \text{ mmHg}$; however, $3–4$ min later, mean arterial blood pressure normalized to the starting value and fell statistically not significantly to $109 \pm 3 \text{ mmHg}$ at the end of the experiments (no differences between groups).

$\text{PaO}_2$ and $\text{PaCO}_2$, as well as $p\text{H}$, did not change over time or between groups [overall means (during ventilation with air): $\text{PaO}_2 95 \pm 2 \text{ mmHg}$; $\text{PaCO}_2 40 \pm 1 \text{ mmHg}$; $p\text{H} 7.41 \pm 0.01$].

The plasma concentration of glucose ($7.9 \pm 0.2 \text{ mmol/l}$) did not change during the experiments and did not differ between the experimental groups.

The $\text{Po}_2$ in the venous blood of the sagittal sinus was $41.5 \pm 0.3 \text{ mmHg}$ under control conditions ($n = 3$). Immediately after initiation of increased ICP, the $\text{Po}_2$ in the blood of the sagittal sinus decreased statistically significantly to $26.9 \pm 3.7 \text{ mmHg}$ ($n = 5$).

Renal blood flow was not influenced by the induction of increased (i)ICP (renal blood flow before iICP, $5.44 \pm 1.10 \text{ ml/min}$; RBF during iICP, $5.57 \pm 1.14 \text{ ml/min}$; $n = 4$ each).
DISCUSSION

The present study was done to clarify whether the oxygen-sensitive sensor, which triggers the renal synthesis of Epo, is exclusively located within the kidney, as has been proposed in the literature up to now. We examined the hypothesis whether intrarenal Epo production is also influenced by extrarenal mechanisms.

Previous data were obtained from experiments in rats in which a reduced supply of oxygen in the kidneys was achieved by experimental stenoses of the renal arteries. However, the results clearly show that this condition causes only a very slight (33) or no (3) increase of Epo production. Similar experiments in dogs or rabbits led to no or only very slight elevations in Epo plasma levels (9, 18, 22) or even to their reduction (23).

These inconsistent results prompted us to question the theory that the Epo-regulating oxygen-sensitive sensor is located primarily in the kidney.

Experiments in isolated perfused rat kidney have shown that, after a clear reduction of the PO2 of the perfusion medium, the Epo production rate is elevated to some extent (27). If, on the other hand, the whole animal is exposed to hypobaric hypoxia, the Epo production rate is, despite an identical renal oxygen supply, ~22 times higher than in the isolated kidney (28).

A possible role of the efferent autonomous nervous system in the regulation of the synthesis of Epo was already proposed some time ago (8). However, the theory of nervous control of Epo production was disproved by the fact that kidney transplant patients already have normal regulation of Epo synthesis a few days after a successful transplant (34). In addition, experiments in rats have shown that experimental derangement of the kidney has no consequence for the renal Epo production rate (5).

However, as indicated by experiments in rats in which isovolemic exchange transfusions of blood were done (29), there is some reason to believe that extrarenal humoral factors exist. Donor rats were exposed to hypobariic hypoxia for 30 min. The blood of these exhypoxic animals was transmitted to untreated normoxic animals, which exhibited increased Epo concentrations in plasma 2–4 h later. In the case of hypophysectomy of the donor rats before their hypoxic exposition, this effect failed to appear. Hence, the hypophysis seems to play a crucial role in the regulation of the synthesis of Epo.

Further evidence for a control of Epo production by the central nervous system can be deduced from experiments in rabbits and monkeys in which electrical stimulations of the hypothalamic regions were done (16). It is known from extensive investigations in cats and dogs that the brain is provided with sensors that are very sensitive to both hypo- and hyperoxia (10) and hypo- and hypercapnia (11).

In the present study, an experimental model was established that made it possible to expose the brain, especially the brain stem, of a rat to hypoxia, the remainder of the organism continuing to be normoxic. This was achieved by increasing the cerebral hydrostatic pressure of a normoxically ventilated animal.

When the cerebral pressure is higher than the mean arterial blood pressure, the transport of oxygen across the blood-brain barrier is reduced or interrupted. This is followed by a clear hypoxia in the cerebrospinal fluid and, consequently, in the tissue of the brain (12, 21, 24). Due to the smallness of the skull of the rat, a reliable measurement of the PO2 in the cerebrospinal fluid of the rat is not possible with commercially available equipment (W. Fleckenstein, unpublished observation).

Measurements in the venous blood taken from the sagittal sinus of the rats showed a clear reduction of the PO2 after increasing the ICP. This is in agreement with data of the literature showing a distinct negative correlation between ICP and PO2 in the tissue of the brain (6, 31, 32).

The results of our measurements of immunoreactive Epo in plasma have shown that elevation of the intracranial hydrostatic pressure led to an elevation of the plasma level of Epo, especially when the ICP was above the level of the mean arterial blood pressure or when the elevation of the ICP persisted for >10 min.

The elevations in Epo plasma levels were not caused by inadequate ventilation of the animals. The effectiveness of the ventilation was confirmed by blood gas analysis once an hour. Moreover, experiments in animals ventilated with pure oxygen showed no differences from experiments in animals ventilated with air.

Increasing the ICP had no influence on renal blood flow. In addition, as was shown by earlier studies from our laboratory, renal blood flow has only minimal effects on renal synthesis of Epo (25, 26).

The effect of the elevation of ICP was specifically aimed at the renal synthesis of Epo. The Epo plasma levels remained at control levels in the animals after bilateral nephrectomy. The remnant Epo in nephrectomized animals derives from extrarenal origins. In particular, the liver, the main source of Epo in the fetus, is perpetually able to synthesize small amounts of Epo (7). However, the adult liver is not able to respond to oxygen deficiency with adequately increased Epo production (7).

Hypophysectomy of the animals likewise abolished the effect of an increase in ICP on renal synthesis of Epo. This finding is in agreement with data from the literature (17) and points to the important role of the hypophysis in conjunction with the renal Epo synthesis.

To test whether the pressure and, hence, hypoxic stress on cerebral structures is specific for an enhancement of renal Epo production, experiments were performed by making use of another kind of stress. The most important stressor for rodents is restriction of their mobility (2). However, the results of our study have shown that this had no consequence on the production rate of Epo.

Systemic parameters like heart rate, blood pressure, concentrations of blood gases, or plasma levels of glucose were not influenced by the cerebral-pressure maneuvers.

In summary, the present results allow the following hypothesis. The renal synthesis of Epo is influenced, at least in part, by extrarenal mechanisms. Due to oxygen deficiencies, cerebral oxygen-sensitive sensors cause the release of one or more “Epo-releasing factors.” These factors reach the kidneys via the blood stream and stimulate the renal synthesis of Epo.

Future experiments will have to show in detail the regulation of renal synthesis of Epo under the support of the brain, especially the brain stem. As a priority, the biology and function of the cerebral “Epo-releasing factors” will have to be clarified by making use of the methods of molecular biology.
ACKNOWLEDGMENTS

We thank Hannelore Dünnbeil, Sylva Dürkop, and Urszula Frackowski for skilful technical assistance. We also thank Gabriela Fleckinger, who did the artwork.

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