0013-7227/99/$03.00/0
Endocrinology
Copyright © 1999 by The Endocrine Society
Vol. 140, No. 2
Printed in U.S.A.

Effects of Pro- and Antioxidative Compounds on Renal Production of Erythropoietin*

IMKE NEUMCKE, BENJAMIN SCHNEIDER, JOACHIM FANDREY, AND HORST PAGEL

Institute of Physiology, Medical University, Luebeck, Germany

ABSTRACT

The most important stimulus for the enhanced synthesis of erythropoietin (Epo) is a lowered O_2 tension in the tissue. However, the mechanism by which an impaired O_2 supply is transduced into appropriate Epo production is still not fully understood. Recently, studies in human hepatoma cells (line HepG2) indicate that reactive O_2 species are involved in the signal transduction from the cellular O_2 sensor to the Epo gene. To clarify the role of reactive O_2 species in the regulation of Epo synthesis in the kidney, the principal Epo-producing organ *in vivo*, we investigated the influence of potent pro- and antioxidants on Epo production in isolated perfused rat kidneys. Under normoxic conditions, the iron chelator desferrioxamine and the antioxidant vitamin A increased renal Epo production, mimicking hypoxic induction. In contrast, supplementation of the perfusion medium of hypoxically perfused kidneys with the prooxidant compounds H_2O_2 or pyrogallol caused a significant reduction of Epo synthesis. The inhibition of Epo formation by reactive O_2 species could be completely antagonized by desferrioxamine and the hydroxyl radical-($OH^{\cdot}$)-scavenger tetramethylthiourea. Vitamin A also antagonized the H_2O_2-dependent inhibition of hypoxically induced Epo synthesis. Interestingly, the addition of the antioxidant vitamin A to hypoxically perfused kidneys also induced Epo production significantly. Our data strongly support the idea that reactive O_2 species, especially H_2O_2, are part of the signaling chain of the cellular O_2-sensing mechanism regulating the renal synthesis of Epo. (*Endocrinology* **140:** 641–645, 1999)

THE GLYCOPROTEIN hormone erythropoietin (Epo) is the principal regulator of the proliferation and differentiation of erythroid progenitors in bone marrow. In adults, Epo is mainly produced in the kidneys. The most important stimulus for an enhanced production of Epo is a lowered oxygen (O_2) supply to the tissue as a result of anemia or hypoxia. However, the mechanism by which changes in O_2 tension are transduced into an appropriate renal Epo production is still not fully understood.

Specific hemoproteins have been proposed as the O_2-binding part of the O_2 sensor (1). Previous *in vitro* studies in cultures of the human hepatoma cell line HepG2 revealed an important role of membrane-bound and/or microsomal *b*-type cytochromes (2, 3). Similar to the NADPH-oxidase from phagocytes (4), the cytochromes generate low, nondamaging amounts of reactive O_2 species dependening on the cellular O_2 tension. These O_2 species may oxidize sulfhydryl groups of regulatory peptides and therefore serve as signaling molecules between the O_2 sensor and the transcriptional machinery of the Epo gene. Referring to this, the small, noncharged, and thus freely diffusible hydrogen peroxide (H_2O_2) is a very attractive candidate. In addition, H_2O_2 has a relatively long biological half-life and is far less cytotoxic than other reactive O_2 species (5, 6).

From the studies in HepG2 cells the hypothesis was put forward that high cellular levels of H_2O_2 under normoxia suppress Epo synthesis, whereas low levels under hypoxia allow full scale Epo gene expression (7, 8). Hence, H_2O_2 could act as a second messenger, possibly influencing cytosolic transcription factors that bind to regulatory sequences of the Epo gene. In fact, hypoxia-induced expression of the Epo gene is critically dependent on the activation of an enhancer element located 3′ of the Epo gene. To this enhancer binds a protein complex termed "hypoxia-inducible factor-1" (HIF-1), which is composed of the two subunits HIF-1α and HIF-1β (9, 10). Only recently, it has been found that H_2O_2 reduces HIF-1 DNA-binding activity under hypoxic conditions by destabilizing the α-subunit (11). Strong reducing agents, however, increase HIF-1 levels by inhibiting the proteasome-dependent degradation of the α-subunit (12).

The present investigation was performed to determine whether this hypothesis also fits the Epo production in the kidney, the predominant site of Epo synthesis after birth. The experiments were performed with isolated serum-free perfused rat kidneys. Levels of secreted Epo and Epo messenger RNA (mRNA) levels in kidney tissue were measured during normoxic and hypoxic perfusion after the addition of H_2O_2 and/or H_2O_2-generating and -scavenging compounds.

Materials and Methods

Kidney perfusion

Right kidneys from adult male Sprague-Dawley rats were perfused as described in detail previously (13). In brief, the kidneys were perfused at constant pressure (100 mm Hg) in a recirculation system for 3 h. The perfusion medium was a substrate- and amino acid-enriched Krebs-Henseleit buffer supplemented with predialyzed (three periods of 3 h each) BSA (60 g/liter; Biomol, Hamburg, Germany) and freshly drawn and washed human erythrocytes (5%). To ensure stable organ function during the experiments, the perfusion medium was dialyzed against a 25-fold volume of a protein- and cell-free solution. The dialyzer also served for oxygenation of the perfusion medium by bubbling prewarmed and moistened gas mixtures through the dialysate. The arterial

Received July 14, 1998.

Address all correspondence and requests for reprints to: Horst Pagel, Ph.D., Institute of Physiology, Medical University, Ratzeburger Allee 160, 23538 Luebeck, Germany. E-mail: pagel@physio.mu-luebeck.de.

* This work was supported by the Deutsche Forschungsgemeinschaft (SFB 367/C8). The paper presents parts of the M.D. theses of I.N. (protein data) and B.S. (mRNA data).

Downloaded from https://academic.oup.com/endo/article/140/2/641/2990331 by guest on 21 August 2022

pO_2 was 149.0 ± 1.8 mm Hg during normoxic and 26.3 ± 0.9 mm Hg during hypoxic perfusion. The arterial pH was 7.4 throughout. During the different experimental series one of the following agents or a combination of them were added to the perfusion medium before starting the isolated perfusion (the final concentrations are given in *parentheses*): hydrogen peroxide (H_2O_2; 100 μmol/liter), pyrogallol (1,2,3-trihydroxybenzene; 100 μmol/liter), desferrioxamine mesylate (DFO; 100 μmol/liter), 1,1,3,3-tetramethyl-2-thiourea (TMTU; 1 mmol/liter), and vitamin A (retinol acetate; 0.5 mg/liter). The concentrations were chosen according to redox experiments in HepG2/Hep3B cell culture systems (8, 14). All substances were obtained from Sigma Chemical Co. (Deisenhofen, Germany), except TMTU, which was obtained from Fluka (Neu-Ulm, Germany).

Determination of the fractional sodium reabsorption

The sodium concentrations in urine and perfusate were determined by flame photometry (AFM 5051, Eppendorf, Hamburg, Germany). The fractional sodium reabsorption was calculated as the ratio of the sodium reabsorption rate to the sodium filtration rate.

Epo measurements

Epo was measured in duplicate by an enzyme-linked immunoassay in samples of the perfusion medium. The assay was performed according to the instructions of the manufacturer (Medac, Hamburg, Germany), except that rat serum Epo previously calibrated by bioassay was used as the standard instead of human Epo.

RNA extraction and Epo mRNA quantitation

At the end of the perfusion period, kidneys were weighed, snap-frozen in liquid nitrogen, and subsequently homogenized in guanidinium thiocyanate solution (4 mol/liter with 0.1 mol/liter β-mercaptoethanol) using a Polytron homogenizer (Kinematica, Luzern, Switzerland) at setting 10 for 20 s. From 700 μl of the homogenate, total RNA was extracted using the acidic phenol-chloroform method (15). After redissolving the RNA in diethylpyrocarbonate-treated water, the concentration was determined by measuring the absorbance at 260 nm. To check the integrity of the RNA, aliquots were run on a 1.1% formaldehyde/agarose gel. Five micrograms of total RNA were reverse transcribed into first strand complementary DNA (cDNA) using oligo(deoxythymidine)$_{15}$ as primer for the reverse transcriptase Moloney murine leukemia virus (Promega Corp., Heidelberg, Germany). The total volume of the reaction was 25 μl. RT was performed at 42 C for 60 min after an initial denaturation step at 68 C for 10 min. The reaction was terminated by boiling the samples for 10 min. Until quantitation by competitive PCR, cDNA stocks were kept at −20 C. All RNA samples were run in one RT reaction to minimize differences in RT efficiency, which was less than or equal to 5%, as determined previously (16).

Quantitation of cDNA was achieved by two PCR methods. First, the TaqMan system (PE Applied Biosystems, Weiterstadt, Germany) was used according to the manufacturer's instructions. Primers were selected by the software provided by PE Applied Biosystems for TaqMan and had the following sequences: upstream, 5′-CTCCG-AACACTCACAGCGG-3′; downstream, 5′-GGTCACCTGTCCCC-TCTCCT-3′; and internal hybridization oligo, 5′-CGGGTCTACTC-CAACTTCCTCCGGG-3′. The reaction temperatures were 95 and 55 C.

Second, a competitive PCR was performed as described previously (16). Each sample was checked for possible DNA contamination. Competitive PCR led to the same mean values, albeit a higher SE due to the lower resolution of this method.

Statistics

The data were normalized to 1 g kidney wet weight (based on the weight of the left, nonperfused kidney) and are given as the mean ± SEM. The results of Epo mRNA quantitation are expressed as the percentage of Epo mRNA in hypoxically perfused rat kidneys and are the mean ± SEM of the data obtained with the TaqMan system. To compare Epo production during hypoxia with that during normoxia, a two-way ANOVA was performed. Within the factor time, Helmert contrasts were calculated (multiple ANOVA in SPSS-X). Dunnett's test was applied to compare a control mean with several treatment means. $P < 0.05$ was set as the significance level.

Results

Figure 1 shows that Epo production in the isolated rat kidney continuously increased during the 3 h of normoxic perfusion up to 198 ± 28 mU/g kidney (n = 5). Hypoxic perfusion led to significantly increased Epo production of up to 481 ± 38 mU/g kidney (n = 9).

The addition of the antioxidants DFO and vitamin A increased Epo production under normoxia (Fig. 2) almost to levels achieved by hypoxic perfusion (see Fig. 3). Both substances roughly doubled the normoxic renal Epo production (controls, 198 ± 28; DFO, 400 ± 31; vitamin A, 374 ± 38 mU/g kidney; n = 6 each; Fig. 2). Under these normoxic conditions, H_2O_2 significantly diminished Epo production from 198 ± 28 mU/g kidney (controls; n = 5) to 90 ± 11 mU/g kidney (H_2O_2 treated; n = 6; not shown)

Hypoxia-induced Epo production during the 3-h perfusion period was significantly reduced from 481 ± 38 mU/g kidney (controls; n = 9) to 342 ± 49 mU/g kidney (n = 5) by the addition of H_2O_2 or stimulation of endogenous H_2O_2-formation by the superoxide anion ($O_2^{-}\cdot$)-generating substance pyrogallol (391 ± 32 mU/g kidney; n = 5; Fig. 3). The H_2O_2-induced inhibition of Epo formation was completely antagonized by the iron chelator DFO (H_2O_2 plus DFO, 490 ± 20 mU/g kidney; n = 4) and the hydroxyl radical ($OH^{\cdot}$) scavenger tetramethylthiourea (H_2O_2 plus TMTU, 475 ± 44 mU/g kidney; n = 5; Fig. 3). As expected, DFO alone had no effect on Epo production of the isolated hypoxically perfused rat kidney (464 ± 12 mU/g kidney; n = 4; not shown). The effects on Epo protein were the result of an altered Epo gene expression, as the addition of H_2O_2 significantly reduced Epo mRNA levels that were antagonized by DFO and TMTU (Table 1).

Interestingly, treatment with the antioxidant vitamin A significantly increased hypoxia-induced Epo production to

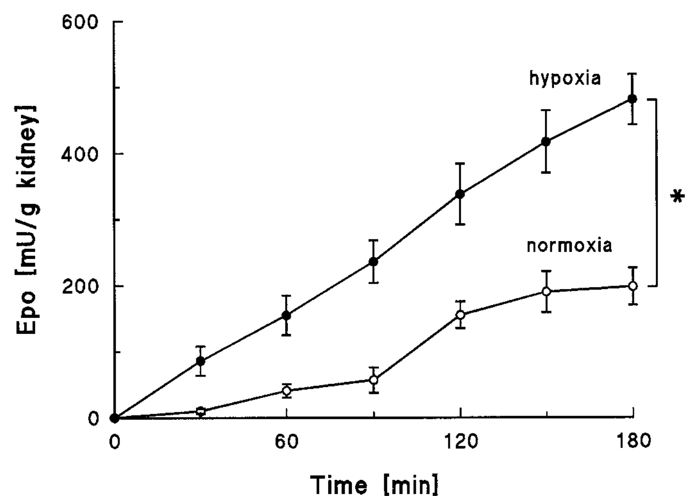

FIG. 1. Epo production rates in the isolated perfused rat kidney during normoxic (*open circles*; n = 5) and hypoxic (*filled circles*; n = 9) perfusion. The mean ± SEM are shown. *, $P < 0.05$ for the Epo time courses (by ANOVA with repeated measurements; first factor, pO_2; second factor, time).

Downloaded from https://academic.oup.com/endo/article/140/2/641/2990331 by guest on 21 August 2022

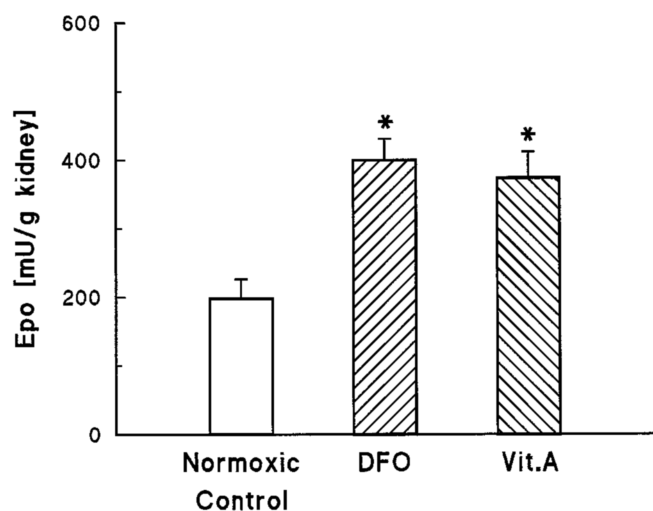

FIG. 2. Effects of DFO (n = 6) and vitamin A (Vit.A; n = 6) on the 3-h Epo production rates during normoxic perfusion of the isolated rat kidney. The mean ± SEM are shown. *, $P < 0.05$ *vs.* controls (by Dunnett's test; n = 5).

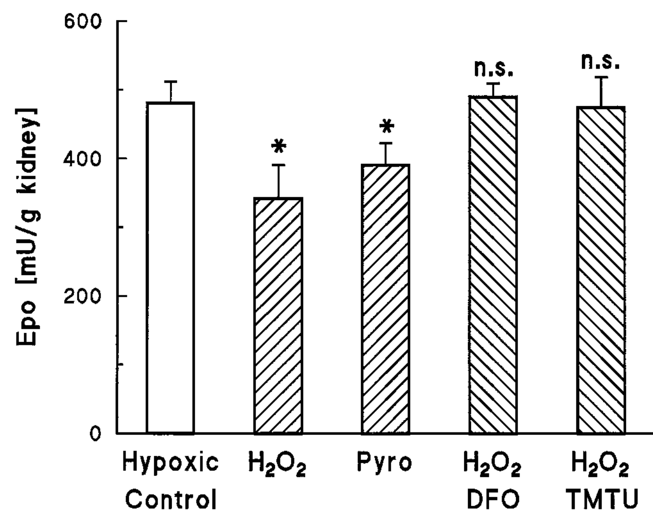

FIG. 3. Inhibition by hydrogen peroxide (H_2O_2; n = 5) and pyrogallol (Pyro; n = 5) of the hypoxically induced 3-h production rates of Epo in the isolated perfused rat kidney. DFO (H_2O_2 plus DFO; n = 4) and TMTU (H_2O_2 plus TMTU; n = 5) antagonized the inhibition caused by H_2O_2. The mean ± SEM are shown. *, $P < 0.05$; n.s., $P > 0.05$ (*vs.* controls, by Dunnett's test; n = 9).

682 ± 12 mU/g kidney (n = 8; Fig. 4). Vitamin A was also able to antagonize the inhibition of Epo production induced by H_2O_2 (474 ± 82 mU/g kidney; n = 6; Fig. 4).

As judged by the fractional renal sodium reabsorption, the exocrine function of the isolated perfused rat kidney was not influenced by the addition of pro- or antioxidative compounds to the perfusion medium (normoxic controls, 92 ± 1%; treatment groups, 89–93%; hypoxic controls, 60 ± 3%; treatment groups, 52–63%; not shown).

Discussion

Isolated rat kidneys perfused with a serum-free perfusion medium produce Epo in a pO_2-dependent manner. Together with previous results from our laboratory (13, 14) and others (17, 18), this experimental set-up appears to be suitable to study the mechanisms of pO_2-dependent Epo synthesis.

TABLE 1. Effects of H_2O_2 alone, H_2O_2 plus desferrioxamine (DFO), and H_2O_2 plus tetramethylthiourea (TMTU) on the Epo mRNA levels in hypoxically perfused rat kidneys

	Hypoxic control	H_2O_2	H_2O_2 + DFO	H_2O_2 + TMTU
Epo mRNA	100	60 ± 1[a]	84 ± 13	82 ± 2

Values are the mean ± SEM, expressed as a percentage of the control value (n = 4–5).

[a] $P < 0.05$ *vs.* control (by Dunnett's test).

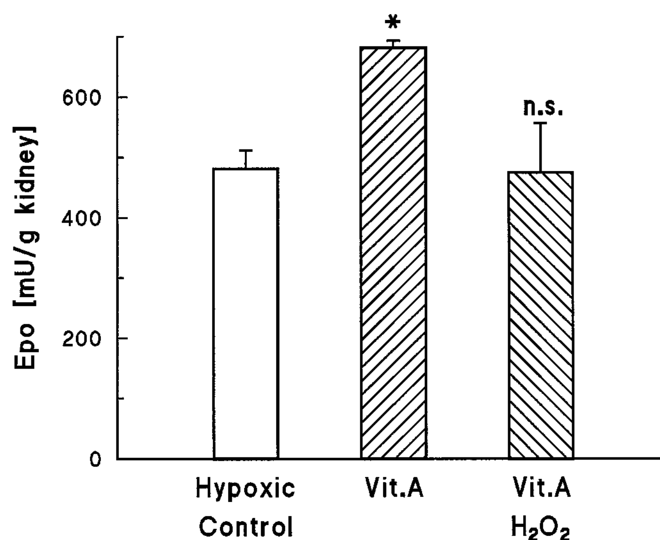

FIG. 4. Effects of vitamin A (Vit.A; n = 8) and Vit.A plus H_2O_2 (n = 6) on the hypoxically induced 3-h production rates of Epo in the isolated perfused rat kidney. The mean ± SEM are shown. *, $P < 0.05$; n.s., $P > 0.05$ (*vs.* controls, by Dunnett's test; n = 9).

The addition of 5% erythrocytes to the perfusion medium improves kidney function (19), but increasing the hematocrit of more than 5% shows no further improvement (20). It is important to note that the Epo production rate in the isolated perfused kidney is independent of the concentration of erythrocytes in the perfusion medium (13).

To check a possible nephrotoxicity of the used pro- and antioxidative compounds in the chosen concentrations, the fractional renal sodium reabsorption, a very sensitive marker for an intact renal metabolism, was determined. Under both normoxic and hypoxic conditions, the fractional sodium reabsorption of the treatment groups was not statistically different from that of the respective controls, indicating a normal function of the isolated perfused rat kidney.

The aim of the present study was to get more insights into the mechanisms by which a variation in the renal O_2 supply is transduced into an appropriate Epo production. Recently, reactive O_2 species at low, nontoxic concentrations have been recognized as intracellular signaling molecules (21). The human hepatoma cell line HepG2 produces H_2O_2 depending on the pericellular pO_2. The highest amounts of secreted Epo in the culture supernatant were found at pO_2 values where H_2O_2 production was lowest (7). However, as in adult life the kidneys are the primary site for Epo synthesis, we studied the effect of redox-modifying agents of Epo production in isolated perfused rat kidneys.

Downloaded from https://academic.oup.com/endo/article/140/2/641/2990331 by guest on 21 August 2022

The addition of the antioxidants DFO or vitamin A to the normoxic perfusion medium increased renal Epo synthesis and almost completely mimicked hypoxia. This would be in line with the hypothesis that reactive O_2 species, especially H_2O_2, suppress Epo production under normoxic conditions. Under hypoxia, when reduced endogenous production of reactive O_2 species allows full scale Epo production, both exogenous H_2O_2 and the stimulation of endogenous H_2O_2 formation by pyrogallol significantly suppressed the rate of Epo production. The iron chelator DFO or the hydroxyl scavenger TMTU completely antagonized the H_2O_2-induced suppression of the Epo production in the isolated perfused rat kidney. These Epo protein data reflected the respective data of the Epo mRNA levels in kidney tissue.

Our data suggest that reactive O_2 species control renal Epo production and thus participate in the O_2-sensing process in the kidney. The results herein from experiments with isolated kidneys corroborate similar data that have been obtained from cell culture studies with HepG2 hepatoma cells (7). Thus, H_2O_2 seems to be an attractive candidate for a signaling molecule between the renal O_2 sensor and the transcriptional activator(s) of the Epo gene. As the suggested mode of action of DFO is an interference with the decomposition of H_2O_2 to $OH^{\cdot}$ (Fenton reaction) (8), it was important to see this antagonistic effect in the kidney as well. Moreover, it appears reasonable to assume that the DFO-dependent induction of Epo production under normoxic conditions is also due to the inhibition of hydroxyl radical production from endogenously produced H_2O_2. Thus, DFO may mimic hypoxia in human hepatoma cells and rat kidneys without removing iron from putative hemeproteins (1). In a very recent publication, Srinivas *et al.* (22) reported that HIF-1α itself appears to be a nonheme iron protein. At least within hepatoma cells, hydroxyl radicals are preferentially detected in close vicinity of the nucleus (23), and one may speculate that they are generated from H_2O_2 in the presence of iron proteins such as HIF-1α. A local Fenton-type reaction would explain the reduced stability of HIF-1α-protein upon H_2O_2 treatment (11).

Therefore, the lack of effect of DFO under hypoxic conditions, when little H_2O_2 is generated by the kidney, was less surprising than the H_2O_2-dependent inhibition of Epo production at perfusion with high pO_2. After our hypothesis even under these normoxic conditions one can expect some HIF-1 activation, which was abolished by the addition of H_2O_2.

Under hypoxic conditions the production of Epo in the isolated perfused rat kidney significantly increased when vitamin A was added to the perfusion medium. Carotenoids are considered antioxidants because of their capacity to scavenge reactive O_2 species (24). The vitamin A-induced increase in Epo production disappeared after the addition of H_2O_2. However, unlike DFO, vitamin A also increased Epo synthesis under normoxic and hypoxic conditions. This finding is in line with the results of corresponding experiments in Hep3B and HepG2 cells (16, 25). Okano *et al.* have proposed that vitamin A activates Epo gene transcription, because Epo mRNA levels are elevated in vitamin A-treated HepG2 cell cultures (25). As vitamin A, in addition to its ability to scavenge reactive O_2 species (24), also binds to retinoic acid receptors, one may speculate that its action is mediated through binding to the steroid-responsive element (DR-2 sequence) in the 3′-enhancer region of the Epo gene (25).

Based on the findings presented herein together with the current knowledge from the literature, it can be proposed that H_2O_2 acts as a negative signaling molecule connecting O_2-sensitive hemeproteins with the Epo gene transcription factor(s). The lack of H_2O_2 in hypoxia allows for an increased Epo gene transcription. Possibly, HIF-1α may be the oxygen sensor and/or the effector of H_2O_2 function. Moreover, our data are in line with previous reports of the important role of H_2O_2 in other O_2 sensor systems, such as in preparations of the carotid body (26), pulmonary neuroepithelial bodies (27), or pulmonary resistance vessels (28).

Acknowledgment

We acknowledge the excellent technical assistance of Mrs. Urszula Frackowski. In addition, we like to thank PE Applied Biosystems (Weiterstadt, Germany) for their support in setting up the TaqMan system.

References

1. **Goldberg MA, Dunning SP, Bunn HF** 1988 Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science 242:1412–1415
2. **Fandrey J, Seydel FP, Siegers CP, Jelkmann W** 1990 Role of cytochrome P_{450} in the control of the production of erythropoietin. Life Sci 47:127–134
3. **Görlach A, Holtermann G, Jelkmann W, Hancock JT, Jones AS, Jones WTG, Acker H** 1993 Photometric characteristics of haem proteins in erythropoietin-producing hepatoma cells (HepG2). Biochem J 290:771–776
4. **Bachmann S, Ramasubbu K** 1997 Immunohistochemical colocalization of the α-subunit of neutrophil NADPH oxidase and ecto-5′-nucleotidase in kidney and liver. Kidney Int 51:479–482
5. **Chance B, Sies H, Boveris A** 1979 Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605
6. **Matthew BG, McCord JM** 1986 Chemistry and cytotoxicity of reactive oxygen metabolites. In: Taylor AE, Matalon S, Ward PA (eds) Physiology of Oxygen Radicals. American Physiological Society, Bethesda, chapt 1:1–18
7. **Fandrey J, Frede S, Jelkmann W** 1994 Role of hydrogen peroxide in hypoxia-induced erythropoietin production. Biochem J 303:507–510
8. **Fandrey J, Frede S, Ehleben W, Porwol T, Acker H, Jelkmann W** 1997 Cobalt chloride and desferrioxamine antagonize the inhibition of erythropoietin production by reactive oxygen species. Kidney Int 51:492–496
9. **Wang GL, Jiang BH, Rue EA, Semenza GL** 1995 Hypoxia-inducible factor 1 is a basic-helix-loop-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 92:5510–5514
10. **Wang GL, Jiang BH, Semenza GL** 1995 Effect of altered redox state on expression and DNA-binding activity of hypoxia-inducible factor 1. Biochem Biophys Res Commun 212:550–556
11. **Huang LE, Arany Z, Livingston DM, Bunn HF** 1996 Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its α subunit. J Biol Chem 271:32253–32259
12. **Salceda S, Caro J** 1997 Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. J Biol Chem 272:22642–22647
13. **Pagel H, Jelkmann W, Weiss C** 1991 Isolated serum-free perfused rat kidneys release immunoreactive erythropoietin in response to hypoxia. Endocrinology 128:2633–2638
14. **Jelkmann W, Pagel H, Hellwig T, Fandrey J** 1997 Effects of antioxidant vitamins on renal and hepatic erythropoietin production. Kidney Int 51:497–501
15. **Chomczinski P, Sacci N** 1987 Single step method of RNA isolation by acid guanidiniumthiocyanat-phenol-chloroform-extraction. Anal Biochem 162:156–159
16. **Fandrey J, Bunn HF** 1993 In vivo and in vitro regulation of erythropoietin mRNA: measurement by competitive polymerase chain reaction. Blood 81:617–623
17. **Ratcliffe PJ, Jones RW, Phillips RE, Nicholls LG, Bells JI** 1990 Oxygen-dependent modulation of erythropoietin mRNA levels in isolated rat kidneys studied by RNAse protection. J Exp Med 172:657–660
18. **Scholz H, Schurek HJ, Eckardt KU, Kurtz A, Bauer C** 1991 Oxygen-dependent erythropoietin production by isolated perfused rat kidney. Pflugers Arch 418:228–233

Downloaded from https://academic.oup.com/endo/article/140/2/641/2990331 by guest on 21 August 2022

19. **Swanson JW, Besarab A, Pomerantz PP, DeGuzman A** 1981 Effect of erythrocytes and globulin on renal functions of the isolated rat kidney. Am J Physiol 56:353–358
20. **Pagel H, Stolte H** 1992 On the glomerular mechanism of renal protein excretion. Renal Physiol Biochem 15:249–256
21. **Khan AU, Wilson T** 1995 Reactive oxygen species as cellular messengers. Chem Biol 2:437–445
22. **Srinivas V, Zhu X, Salceda S, Nakamura R, Caro J** 1998 Hypoxia-inducible factor 1α (HIF-1α) is a non-heme iron protein. J Biol Chem 273:18019–18022
23. **Porwol T, Ehleben W, Zierold K, Fandrey J, Acker H** 1998 The influence of nickel and cobalt on putative members of the oxygen-sensing pathway of erythropoietin-producing HepG2 cells. Eur J Biochem 256:16–23
24. **Yu BP** 1994 Cellular defenses against damage from oxygen species. Physiol Rev 74:139–162
25. **Okano M, Masuda S, Narita H, Masushige S, Kato S, Imagawa S, Sasaki R** 1994 Retinoic acid up-regulates erythropoietin production in hepatoma cells and in vitamin A depleted rats. FEBS Lett 349:229–233
26. **Cross AR, Henderson L, Jones OTG, Delpiano MA, Hentschel J, Acker H** 1990 Involvement of a NAD(P)H oxidase as a pO_2 sensor protein in the rat carotid body. Biochem J 272:743–747
27. **Youngson C, Nurse C, Yeger H, Cutz E** 1993 Oxygen sensing in airway chemoreceptors. Nature 365:153–155
28. **Omar HA, Mohazzab KM, Mortelliti MP, Wolin MS** 1993 O_2-dependent modulation of calf pulmonary tone by lactate: potential role of H_2O_2 and cGMP. Am J Physiol 264:L141–L145

7th European Workshop on Pituitary Adenomas
10–13 September 2000, Oxford, UK

Chairman: Professor John Wass. In association with the European Neuroendocrine Association. Further details from Janet Crompton, 29 North Road, St. Andrews, Bristol BS6 5AD, UK. Tel +44 (0)117 924 8160; fax +44 (0)117 924 1208; e-mail: janetcrompton@compuserve.com.

Downloaded from https://academic.oup.com/endo/article/140/2/641/2990331 by guest on 21 August 2022