Role of erythropoietin in adaptation to hypoxia

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Summary. The glycoprotein hormone erythropoietin (EPO) counteracts tissue hypoxia by increasing the systemic oxygen-carrying capacity. It induces augmentation of red blood cell mass by stimulating the formation and differentiation of erythroid precursor cells in the bone marrow.
EPO production is increased under various forms of diminished oxygen supply such as anemic or hypoxic hypoxia. In the adult organism, the kidneys are the major source of EPO. The precise nature of the cells responsible for renal EPO production, however, has not yet been elucidated. Most likely, peritubular cortical cells, e.g. interstitial or endothelial cells, are involved in the elaboration of the hormone. From the observation that isolated perfused rat kidneys produce EPO in an oxygen-dependent fashion we conclude that the 'oxygen sensor' that controls hypoxia-induced EPO synthesis is located in the kidney itself. Within the kidneys, the local venous oxygen tension which reflects the ratio of oxygen supply to oxygen consumption is measured and transformed into a signal that regulates the formation of EPO. However, the mechanism by which a decrease of oxygen delivery to the kidneys is linked to an enhanced EPO gene expression is not yet known. Two possible mechanisms of regulation are discussed: First, renal hypoxia could lead to enhanced formation of metabolic mediators, for example prostaglandins or adenosine, which might stimulate EPO gene transcription by increasing cellular levels of second messenger molecules. Second, some kind of molecular 'oxygen receptor' such as a heme protein, that controls EPO formation by an oxygen-dependent conformational change, could mediate signal transduction.
Key words. Hypoxia; oxygen sensing; erythropoietin; isolated kidneys.

Introduction

Tissue hypoxia is characterized by a disproportion between tissue oxygen supply and tissue oxygen consumption. Since adequate oxygen delivery is essentially maintained by the systemic oxygen-carrying capacity, as determined by the red blood cell mass, the enhanced formation of erythroid precursors in the bone marrow represents an effective mechanism of long-term adaptation to hypoxia. The glycoprotein hormone erythropoietin (EPO), which is produced physiologically by the kidneys, is the main regulator of erythroid cell differentiation and maturation[21]. The physiological role of EPO is to adjust the pool of circulating erythrocytes to the actual oxygen demand[26]. Hence, EPO production is increased under conditions of diminished oxygen supply such as anemia or hypoxia[21]. In this context, the question arises of the precise site of 'oxygen sensing', and of the mechanism of signal transduction by which reduced oxygen delivery induces enhanced EPO production in the kidneys. In the following, we will discuss present concepts of the regulation of EPO formation and give a short overview of the physiology and biochemistry of the hormone.

EPO – Molecular structure and sites of action

EPO is a glycoprotein hormone with a molecular weight of approximately 34,000 Da[28, 31, 41]. Its protein backbone is composed of 165 amino acids, and has a molecular weight of about 18,400 Da[28, 31, 41]. Four complex carbohydrate chains containing a high amount of sialic acid are attached to the protein at three N-linked glycosylation sites and one O-linked one[44]. Deglycosylation of the EPO molecule results in a loss of in vivo bioactivity but not of immunoreactivity[49]. This loss of biological activity in vivo can be ascribed to a higher clearance rate of the deglycosylated form as compared to the intact molecule[49].
The target cell for EPO in the erythropoietic pathway is the colony-forming unit erythroid (CFU-E), a late erythroid precursor in the bone marrow[34, 54]. At physiological levels, EPO increases the mitotic rate of CFU-E and stimulates the proliferation of proerythroblasts[34, 54]. At higher concentrations, EPO also acts on the late burst forming unit erythroid colony cells (BFU-E), which are early erythroid progenitors[54]. Recent studies indicate that the EPO molecule binds to a low and to a high affinity class of EPO receptors. It is thought that the high affinity receptors are the physiologically important ones[5]. A calcium-dependent reaction seems to be involved in the mechanism that initiates cell division when the EPO molecule binds to the receptor on its target cell.[33].

EPO production sites

The classic work demonstrating that the kidneys are the main EPO production site in the adult organism was performed by Jacobson and co-workers in 1957. They were able to show that bilateral nephrectomy prevented a rise in serum EPO levels in rats following bleeding[19]. More direct evidence for renal de novo synthesis of EPO was provided by the demonstration of EPO messenger RNA in kidneys taken from hypoxic and anemic animals[3, 4, 46].

The cellular site of EPO formation within the kidneys, however, is still subject to discussion. Using cDNA probes for EPO [18, 30, 38] several groups of investigators could show that the EPO-producing cells are located in the peritubular interstitium of the kidney cortex [23, 27]. However, further attempts to reveal the nature of these cells with immunohistochemical methods – possible candidates are endothelial cells, macrophages or interstitial cells – failed to provide clear evidence [9, 27]. More detailed information could be obtained in future by combining on the same tissue section in situ hybridization for EPO mRNA with immunohistochemical staining of cell-specific antigens.

Besides the kidneys, the liver, which is the most important source of extrarenal EPO, accounts for approximately 20% of total EPO [15]. However, the liver cannot compensate for a loss of EPO formation by the kidneys, e.g. during chronic renal failure. Nevertheless, it represents the main source of EPO during fetal life [56].

Regulation of EPO formation in vivo

Since levels of circulating serum EPO are elevated in different forms of hypoxia including anemia (decreased oxygen transport capacity) and hypoxic hypoxia (reduced oxygen saturation) it becomes evident that the primary determinant of EPO formation is the oxygen content of the blood [2, 11, 26]. The latter only becomes apparent after the removal of a certain amount of oxygen from the blood during capillary passage. In conclusion, the 'oxygen sensor' that governs renal EPO formation is sensitive to changes in the local venous P_{O_2} [2, 26]. This in turn is not only influenced by the oxygen supply but is also affected by the oxygen consumption, which indicates that the 'oxygen sensor' records changes in the ratio of oxygen delivery to oxygen demand. Hence the following questions arise:

1. Where in the organism is the blood oxygen content measured and transformed into a signal that regulates the elaboration of EPO?
2. Of what kind is the signal transduction mechanism that links decreased oxygen supply to an enhanced EPO production by the kidneys?

Renal versus extrarenal oxygen-sensing sites

Until now, the question whether the sensing of oxygen supply and its transduction into adequate EPO synthesis is performed extrarenally or in the kidney itself is a matter for controversy [2, 36]. The first clinical evidence for a subdiaphragmal localization of the 'oxygen sensor' was provided by Stohlman et al., who described a patient with severe erythrocytosis due to a patent ductus arteriosus with a reversed shunt leading to selective hypoxemia of the lower half of the body [50]. In this context, the 'oxygen sensor' is operationally defined as the oxygen-measuring process that controls renal EPO synthesis. A major argument against an essential oxygen sensing by the kidneys comes from the observation that selective reduction of oxygen supply to the kidneys by renal artery constriction only causes a linear increase of serum EPO levels in rats, whereas the same decrease of oxygen supply caused by reduction of the systemic oxygen transport capacity (exchange-transfusion of blood with plasma) leads to an exponentially increasing EPO production [36]. However, when interpreting these data, it should be remembered that the reduction of renal blood flow not only causes a decrease of oxygen delivery to the kidneys but also decreases tubular sodium load, and thereby leads to a reduction of renal oxygen consumption [6]. Since the rate of renal EPO formation seems to depend more upon the ratio of oxygen supply to the kidneys to oxygen consumption by the kidneys than upon the oxygen delivery alone [8], diminished tubular sodium reabsorption would result in a decreased sensitivity of the 'oxygen sensor' and consequently in a reduction of EPO formation [2, 8].

Strong evidence for an intrarenal localization of the 'oxygen sensor' that controls EPO formation would be obtained from the demonstration that isolated perfused kidneys produce EPO in an oxygen-dependent manner, as is known for the intact organism. For this purpose, we isolated kidneys from normoxic rats and perfused them in a recirculating system at a constant pressure of 100 mm Hg, as described [45]. The perfusion medium consisted of a modified physiological saline containing bovine serum albumin (2 g/dl) and a 10% fraction of freshly-washed human erythrocytes. The perfusate was continuously dialyzed against a 20-fold volume of protein-free Krebs-Henseleit solution. The oxygen supply to the kidneys was adjusted by equilibrating the dialysate with gas mixtures of different oxygen content. The amount of EPO produced by the kidneys was determined by radioimmunoassay in aliquots drawn from the perfusion medium [7]. Using this model we were able to extend previous studies indicating that isolated perfused kidneys release significantly more EPO upon lowering the oxygen tension in the perfusion medium [10, 12, 25, 55]. Furthermore, the maximal amount of EPO released by in vitro perfused kidneys was approximately the same as that calculated for hypoxia-induced EPO production in the intact organism. Since the kidneys contain no stores of EPO [20, 48] the amount of EPO detected in the perfusion medium directly reflects hypoxia-induced de novo synthesis of EPO.

From the observation that isolated kidneys produce EPO in an oxygen-dependent fashion, one might conclude that the 'oxygen sensor' that controls renal EPO formation is located within the kidneys. The precise cellular site of this renal 'oxygen sensor' is yet unknown. Since, as outlined above, renal EPO formation is not only determined by the oxygen supply to the kidneys but also depends upon the oxygen consumption of the kidneys [8], more detailed information on the localization of the intrarenal oxygen-sensing process should be obtained from experiments in which site-specific inhibition of tubular sodium reab-

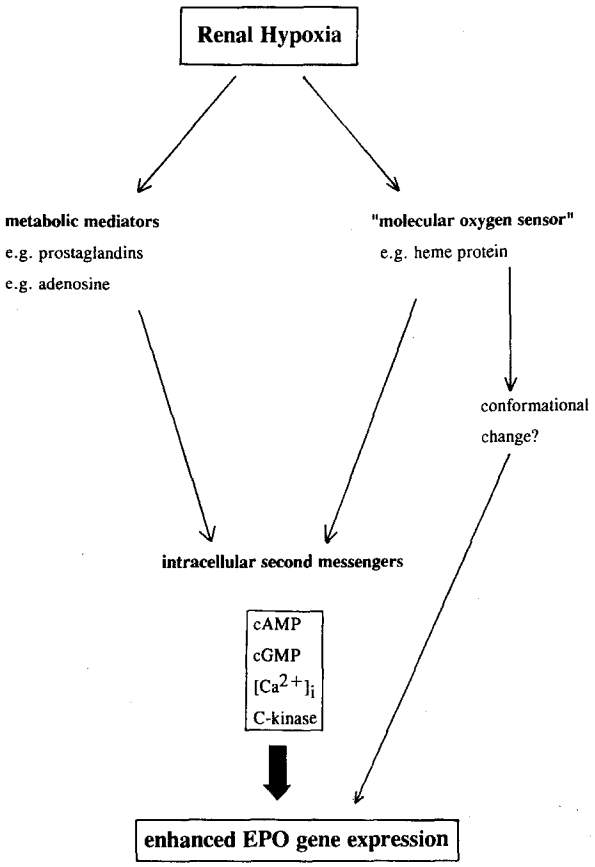
sorption along different segments of the nephron is performed. Recently, it was shown that only the inhibition of *proximal* tubular sodium transport, caused by acetazolamide, significantly reduced hypoxia-induced EPO formation in mice[8]. On the other hand, furosemide, hydrochlorothiazide and amiloride, which inhibit sodium transport predominantly at the thick ascending limb of the loop of Henlé, the early distal tubule, and the late distal tubule and collecting duct, respectively, failed to prevent the rise of EPO production in response to normobaric hypoxia (8 and 14% O_2) and reduced oxygen transport capacity (0.1% carbon monoxide)[8]. These results suggest that the production of EPO is related to proximal tubular sodium reabsorption. It remains to be clarified by future work whether the EPO-producing cells themselves act as 'oxygen sensors' – in this case the rate of proximal tubular sodium transport would modulate the oxygen sensitivity of those cells – or whether the proximal tubule is sensitive to hypoxia, thereby generating some kind of biochemical signal which induces a distinct cell type in its environment to produce EPO.

The process of signal transduction

With regard to the subcellular regulation of EPO formation, strong evidence has been provided that the production of the hormone is at least in part controlled at the level of gene transcription. Firstly, it has been shown that the rise of serum EPO levels of rats exposed to hypoxia is paralleled by a preceding increase in the EPO mRNA content of the kidneys[26]. Furthermore, experiments performed on isolated rat kidney cell nuclei revealed active EPO gene transcription in kidney nuclei from anemic-hypoxic animals, whereas EPO transcriptional activity was not detectable in nuclei from control kidneys[47]. In addition, Koury and co-workers have recently shown that increasing anemia in mice is accompanied by an increase of total renal EPO mRNA content and of serum EPO levels which both correlate with the number of EPO-producing cells in the kidneys[24]. Those findings also indicate that under anemic conditions, additional cells are recruited to maintain adequate EPO synthesis, rather than indicating that solely the amount of EPO produced by an individual cell is increased. However, the mechanism, by which diminished oxygen supply might induce enhanced EPO gene expression is unknown. Nevertheless, the following concepts about the signal transduction mechanism appear rational (fig.):

1. Renal hypoxia might activate a metabolic pathway and thereby lead to increased levels of intracellular second messenger molecules. In analogy to other genes (e.g., human vasoactive intestinal polypeptide gene, and human α-gonadotropin gene)[43], the rate of EPO gene transcription could also be modulated by the cellular content of second messengers.

2. A defined 'oxygen receptor', e.g., a heme protein, might act as 'oxygen sensor' and increase EPO transcriptional activity by an oxygen-dependent change of its conformational state[17].



Schematic diagram illustrating two possible regulatory pathways of EPO formation: 1. Renal hypoxia could activate metabolic mediators such as adenosine or prostaglandins thereby leading to increased cellular levels of second messenger molecules which in turn could regulate EPO gene transcriptional activity. 2. Some kind of 'molecular oxygen sensor', e.g. a heme protein, could modulate expression of the EPO gene by an oxygen-dependent change of its conformation. For further details see text.

Metabolic mediators indicating renal hypoxia

The entirety of the metabolic events following renal hypoxia is much too complex to be considered in this context (for review see Jones[22]). There is some evidence, however, that prostaglandins might be important for the regulation of kidney function during hypoxia[52,53]. An intact prostaglandin system seems to be crucial for adequate EPO formation by the kidneys[13,21]. The mechanism by which the enhanced release of prostaglandins might stimulate EPO production is still elusive. Since it is known that prostaglandins (PGE_2, prostacyclin) activate adenylate cyclase in vivo and in vitro[13,21], one might suggest that the prostaglandins exert their stimulatory effect on EPO formation by increasing the intracellular levels of the classic 'second messenger' cyclic AMP[1,20]. The transcriptional activity of various genes, such as the rat somatostatin, the human α-gonadotropin and the human vasoactive intestinal peptide genes, is known to be

regulated by cyclic AMP[43]. From recent studies on cAMP-dependent control of gene expression strong evidence has been obtained that the catalytic subunit of cAMP-dependent protein kinase can induce transcription of genes containing cAMP-responsive enhancer elements[42]. However, direct experimental support for the theory of cAMP-dependency of EPO gene expression is still missing at the moment. Moreover, since the normal day-to-day elaboration of EPO is so precisely fine-tuned, one would expect more than one single effector system, such as the prostaglandins, to be involved in the control of EPO formation.

A further metabolic indicator for renal hypoxia is the nucleoside adenosine (for review see Osswald[35]). Adenosine is generated in the kidneys by ATP hydrolysis via AMP dephosphorylation[35]. The key enzyme in this process is ecto-5′-nucleotidase, which has been demonstrated to occur in the kidney cortex[29]. In vivo studies as well as hypoxic perfusion of isolated rat kidneys provided strong evidence that renal hypoxia leads to a depletion of tissue ATP that is paralleled by an increased adenosine release from the kidneys[39,40]. Moreover, it was demonstrated in two recent reports that exogenously applied adenosine stimulates EPO formation in hypoxic mice as well as in isolated perfused rat kidneys[37,51]. However, since the intraarterial application of adenosine causes renal vasoconstriction[35] it remains difficult to distinguish whether adenosine directly acts on the EPO-producing cells or indirectly stimulates EPO formation by a reduction of oxygen supply to the kidneys.

Using the isolated perfused rat kidney model described above, we examined in a second series of experiments whether hypoxia-induced EPO production can be mimicked by increasing the cellular levels of classic second messengers. Forskolin (10 μM), which was considered to stimulate adenylate cyclase, thereby increasing cellular cyclic AMP content, significantly decreased renal vascular resistance and caused a more than 20-fold increase in renin release from the kidneys. 8-bromo-cyclic GMP (100 μM), a membrane-permeable analogue of cyclic GMP, also markedly decreased renal vascular resistance. Calmidazolium (1 μM), and W-7 (10 μM), two structurally different putative calmodulin antagonists, were used to inhibit intracellular calcium binding to calmodulin[16]. Although the tested drugs apparently showed biological activity, they all failed to stimulate EPO production in isolated kidneys. Calmidazolium (1 μM) and W-7 (10 μM), however, regularly prevented hypoxia-induced increase of EPO formation in this model without altering renal oxygen consumption. These results suggest that a calcium/calmodulin (Ca/CaM)-dependent reaction is possibly involved in the signal transduction process that controls renal EPO formation. The subcellular mechanism, however, by which Ca/CaM could regulate the generation of EPO remains speculative. Considering that the production of EPO seems to be inversely related to the calcium permeability of the cell membrane[14,32], it would be conceivable that a CaM-dependent Ca^{2+} pump is possibly involved in the signal transduction process.

Molecular 'oxygen receptor' mechanisms

Besides the 'metabolic pathway' described above, it is also possible that renal EPO production is under the control of a 'molecular oxygen receptor'. Very recently, experimental evidence was provided for a very elegant model of 'molecular oxygen sensing'. From experiments performed on a liver carcinoma cell line which produces EPO in an oxygen-dependent fashion, Goldberg and coworkers concluded that a heme protein might regulate EPO synthesis[17]. They proposed that this heme protein would control EPO gene expression by an oxygen-dependent conformational change: At sufficiently low oxygen tensions the heme protein would be in the deoxy or tense conformation and thereby trigger the expression of the EPO gene, whereas the oxy or relaxed state, which would be adopted in conditions of high oxygen partial pressure, is inactive in the stimulation of EPO synthesis[17]. The strongest argument was derived from the observation that carbon monoxide (10%) significantly reduced hypoxia-induced EPO formation in this hepatoma cell line[17]. Carbon monoxide was thought to bind with high affinity to the heme moiety of this protein which would thereby be kept in the oxy conformation and thus prevent hypoxia-induced increase of EPO gene transcription[17]. Elegant though this concept is, it has to be confirmed by the demonstration that a heme protein that controls EPO gene expression by an oxygen-dependent change of its conformation is really present within these cells.

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1 Bauer, C., Metabolic events that may activate erythropoietin production in the hypoxic kidney, in: Oxygen Sensing in Tissues, pp. 93–101. Ed. H. Acker. Springer-Verlag, Berlin 1988.
2 Bauer, C., and Kurtz, A., Oxygen sensing in the kidney and its relation to erythropoietin production. A. Rev. Physiol. *51* (1989) 845–856.
3 Beru, N., Mc Donald, J., Lacombe, C., and Goldwasser, E., Expression of the erythropoietin gene. Molec. cell. Biol. *6* (1986) 2571–2575.
4 Bondurant, M. C., and Koury, M. J., Anemia induces accumulation of erythropoietin mRNA in the kidney and liver. Molec. cell. Biol. *6* (1986) 2731–2733.
5 D'Andrea, A. D., Lodish, H. F., and Wong, G. G., Expression cloning of the murine erythropoietin receptor. Cell *57* (1989) 277–285.
6 Deetjen, P., and Kramer, K., Die Abhängigkeit des O_2-Verbrauchs der Niere von der Na-Rückresorption. Pflügers Arch. *273* (1961) 636–650.
7 Eckardt, K. U., Kurtz, A., Hirth, P., Scigalla, P., Wieczorek, L., and Bauer, C., Evaluation of the stability of human erythropoietin in samples for radioimmunoassay. Klin. Wochenschr. *66* (1988) 241–245.
8 Eckardt, K. U., Kurtz, A., and Bauer, C., Regulation of erythropoietin production is related to proximal tubular function. Am. J. Physiol. *256* (1989) F942–F947.

9 Eckardt, K. U., Möllmann, M., Neumann, R., Brunkhorst, R., Burger, H. U., Lonnemann, G., Scholz, H., Keusch, G., Buchholz, B., Frei, U., Bauer, C., and Kurtz, A., Erythropoietin in polycystic kidneys. J. clin. Invest. *84* (1989) 1160–1166.

10 Erslev, A. J., In vitro production of erythropoietin by kidneys perfused with a serum-free solution. Blood *44* (1974) 77–85.

11 Erslev, A. J., Caro, J., Miller, O., and Silver, R., Plasma erythropoietin in health and disease. Ann. clin. Lab. Sci. *10* (1980) 250–257.

12 Fisher, J. W., and Birdwell, B. J., The production of an erythropoietic factor by the in situ perfused kidney. Acta haemat. *26* (1961) 224–232.

13 Fisher, J. W., Mc Gonigle, R., and Beckman, R., Control mechanisms in kidney erythropoietin production, in: Kidney Hormones, pp. 463–474. Ed. J. W. Fischer. Academic Press, London 1986.

14 Fisher, J. W., Pharmacologic modulation of erythropoietin production. A. Rev. Pharmac. Toxic. *28* (1988) 101–122.

15 Fried, W., The liver as a source of extrarenal erythropoietin. Blood *40* (1972) 671–677.

16 Gietzen, K., Sadorf, I., and Bader, H., A model for the regulation of the calmodulin-dependent enzymes erythrocyte Ca^{2+}-transport AT Pase and the brain phosphodiesterase by activators and inhibitors. Biochem. J. *207* (1982) 541–548.

17 Goldberg, M. A., Dunning, S. P., and Bunn, H. F., Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science *242* (1988) 1412–1415.

18 Jacobs, K., Shoemaker, C., Rudersdorf, R., Neill, S., Kaufmann, J., Mufson, A., Seehra, J., Jones, S. S., Hewick, R., Fritsch, E. F., Kawakita, M., Shimizu, T., and Miyake, T., Isolation and characterization of genomic and cDNA clones of human erythropoietin. Nature *313* (1985) 806–810.

19 Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L., Role of the kidney in erythropoiesis. Nature *179* (1957) 633–634.

20 Jelkmann, W., Temporal patterns of erythropoietin titers in kidney tissue during hypoxic hypoxia. Pflügers Arch. *393* (1982) 88–91.

21 Jelkmann, W., Renal erythropoietin: properties and production. Rev. Physiol. Biochem. Pharmac. *104* (1986) 140–215.

22 Jones, D. P., Renal metabolism during normoxia, hypoxia and ischemic injury. A. Rev. Physiol. *43* (1986) 33–50.

23 Koury, S. T., Bondurant, M. C., and Koury, M. J., Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. Blood *71* (1988) 524–527.

24 Koury, S. T., Koury, M. J., Bondurant, M. C., Caro, J., and Graber, S. E., Quantitation of erythropoietin-producing cells in kidneys of mice by in situ hybridization: correlation with hematocrit, renal erythropoietin mRNA, and serum erythropoietin concentration. Blood *74* (1989) 645–651.

25 Kuratowska, Z., Lewartowski, B., and Michalak, E., Studies on the production of erythropoietin by isolated perfused organs. Blood *18* (1961) 527–534.

26 Kurtz, A., Eckardt, K. U., Tannahill, L., and Bauer, C., Regulation of erythropoietin production. Contr. Nephrol. *66* (1988) 1–16.

27 Lacombe, C., da Silva, J. L., Bruneval, P., Fournier, J. G., Wendling, F., Casadevall, N., Camilleri, J. P., Bariety, J., Varet, B., and Tambourin, P., Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. J. clin. Invest. *81* (1988) 620–623.

28 Lai, P. H., Everett, R., Wang, F. F., Arkawa, T., and Goldwasser, E., Structural characterization of human erythropoietin. J. biol. Chem. *261* (1986) 3116–3121.

29 Le Hir, M., and Kaissling, B., Distribution of 5′-nucleotidase in the renal interstitium of the rat. Cell Tissue Res. *258* (1989) 177–182.

30 Lin, F. K., Suggs, S., Lin, C. H., Browne, J., Smalling, R., Egrie, J. C., Chen, K. K., Fox, G. M., Martin, F., Stabinski, Z., Badrawi, M., Lai, P. H., and Goldwasser, E., Cloning and expression of the human erythropoietin gene. Proc. natl Acad. Sci. USA *82* (1985) 7580–7584.

31 Mc Donald, J. D., Lin, F. K., and Goldwasser, E., Cloning, sequencing, and evolutionary analysis of the mouse erythropoietin gene. Molec. cell. Biol. *6* (1986) 842–848.

32 Mc Gonigle, J. S., Brookins, J., Pegram, B. L., and Fisher, J. W., Enhanced erythropoietin production by calcium channel blockers in rats exposed to hypoxia. J. Pharmac. exp. Ther. *241* (1987) 428–432.

33 Miller, B. A., Scaduto, R. C., Tillotson, D. L., Botti, J. J., and Cheung, J. Y., Erythropoietin stimulates a rise in the intracellular free calcium concentration in single early human erythroid precursors. J. clin. Invest. *82* (1988) 309–315.

34 Nijhof, W., and Wierenga, P. K., Isolation and characterization of the erythroid progenitor cell CFU-E. J. Cell Biol. *96* (1983) 386–392.

35 Osswald, H., Adenosine and renal function, in: Regulatory Function of Adenosine, pp. 399–415. Eds R. M. Berne, T. W. Roll and R. Rubio. Nijhof, London 1983.

36 Pagel, H., Jelkmann, W., and Weiss, C., A comparison of the effects of renal artery constriction and anemia on the production of erythropoietin. Pflügers Arch. *413* (1988) 62–66.

37 Paul, P., Rothmann, S. A., and Meagher, R. C., Modulation of erythropoietin production by adenosine. J. Lab. clin. Med. *112* (1988) 168–173.

38 Powell, J. S., Berkner, K. L., Lebo, R. V., and Adamson, J. W., Human erythropoietin gene: High level expression in stably transfected mammalian cells and chromosome localization. Proc. natl Acad. Sci. USA *83* (1986) 6465–6469.

39 Ramos-Salazar, A., and Baines, A. D., Role of 5′-nucleotidase in adenosine-mediated renal vasoconstriction during hypoxia. J. Pharmac. exp. Ther. *236* (1986) 494–499.

40 Ratcliffe, P. J., Endre, Z. H., Scheinman, S. J., Tange, J. D., Ledingham, J. G. G., and Radda, G. K., ^{31}P nuclear magnetic resonance study of steady-state adenosine 5′-triphosphate levels during graded hypoxia in the isolated perfused rat kidney. Clin. Sci. *74* (1988) 437–448.

41 Recny, M. A., Scoble, H. A., and Kim, Y., Structural characterization of natural human urinary and recombinant DNA-derived erythropoietin. J. biol. Chem. *262* (1987) 17156–17163.

42 Riabowol, K. T., Fink, J. S., Gilman, M. Z., Walsh, D. A., Goodman, R. H., and Feramisco, J. R., The catalytic subunit of cAMP-dependent protein kinase induces expression of genes containing cAMP-responsive enhancer elements. Nature *336* (1988) 83–86.

43 Roesler, W. J., Vandenbark, G. R., and Hanson, R. W., Cyclic AMP and the induction of eucaryotic gene transcription. J. biol. Chem. *263* (1988) 9063–9066.

44 Sasaki, H., Bothner, B., Dell, A., and Fukuda, M., Carbohydrate structure of erythropoietin expressed in Chinese hamster ovary cells by a human erythropoietin cDNA. J. biol. Chem. *262* (1987) 12059–12076.

45 Schurek, H. J., and Alt, J. M., Effect of albumin on the function of perfused rat kidney. Am. J. Physiol. *240* (1981) F569–F576.

46 Schuster, S. J., Wilson, J. H., Erslev, A. J., and Caro, J., Physiologic regulation and tissue localization of renal erythropoietin messenger RNA. Blood *70* (1987) 316–318.

47 Schuster, S. J., Badiavas, P., Costa-Giomi, P., Weinmann, R., Erslev, A. J., and Caro, J., Stimulation of erythropoietin gene transcription during hypoxia and cobalt exposure. Blood *73* (1989) 13–16.

48 Sherwood, J. B., and Goldwasser, E., Extraction of erythropoietin from normal kidneys. Endocrinology *103* (1978) 866–870.

49 Smith Dordal, M., Wang, F. F., and Goldwasser, E., The role of carbohydrate in erythropoietin action. Endocrinology *116* (1985) 2293–2299.

50 Stohlman, F., Rath, C. E., and Rose, J. C., Evidence for a humoral regulation of erythropoiesis. Blood *9* (1954) 712–733.

51 Ueno, M., Brookins, J., Beckman, B., and Fisher, J. W., A_1 and A_2 adenosine receptor regulation of erythropoietin production. Life Sci. *43* (1988) 229–237.

52 Walker, B. R., Diuretic response to acute hypoxia in the conscious dog. Am. J. Physiol. *243* (1982) F440–F446.

53 Walker, B. R., and Fröhlich, J. C., Renal prostaglandins and leucotrienes. Rev. Physiol. Biochem. Pharmac. *107* (1987) 2–72.

54 Yen, Y. P., Zabala, P., Doney, K., et al., Hematopoietic growth factors in human serum. Erythroid burst-promoting activity in normal subjects and in patients with severe aplastic anemia. J. Lab. clin. Med. *106* (1985) 384–392.

55 Zangheri, E. O., Campana, H., Ponce, F., Silva, J. C., Fernandez, F. O., and Suarez, J. R. E., Production of erythropoietin by anoxic perfusion of the isolated kidney of a dog. Nature *199* (1963) 572–573.

56 Zanjani, E. D., Peterson, E. N., Gordon, A. S., and Wasserman, L. R., Erythropoietin production in the fetus: role of the kidney and maternal anemia. J. Lab. clin. Med. *83* (1974) 281–287.

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