

Oxygen-dependent erythropoietin production by the isolated perfused rat kidney

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Abstract. In this study we have investigated the role of oxygen delivery and of classic second messengers on erythropoietin production by the isolated perfused rat kidney. We found that the rat kidney was capable of de novo synthesis of erythropoietin. The erythropoietin production rate was inversely related to the oxygen pressure in the perfusate and increased from 0.17 to 1.85 U erythropoietin $\text{h}^{-1} \text{g kidney}^{-1}$ when arterial PO_2 was lowered from 500 mmHg to 30 mmHg. Addition of forskolin (10 μM) and 8-bromo-cGMP (100 μM) to the perfusate elicited significant effects on the renal vascular resistance, but had no significant effect on erythropoietin production. Hypoxia-induced erythropoietin formation, however, was blocked by calmidazolium (1 μM) and W-7 (10 μM), two structurally different putative calmodulin antagonists. Calmidazolium and W-7 had no effect on other functional parameters of the isolated perfused rat kidney such as flow rate, glomerular filtration rate or sodium reabsorption. Our findings suggest that the oxygen-sensing mechanism that controls renal erythropoietin production is primarily located in the kidney itself. A calcium/calmodulin-dependent cellular reaction could be involved in the signal transduction process.

Key words: Isolated perfused kidney – Radioimmunoassay – Hypoxia – Renal oxygen sensing – cAMP – cGMP – Calmodulin

Introduction

It is accepted that the kidneys are the main site of erythropoietin (EPO) synthesis in the organism [12, 14]. Within the kidneys EPO is most likely produced by cortical peritubular cells, depending on the oxygen supply to the kidneys [15, 18]. Recently, evidence was provided that EPO production is regulated at least in part by the rate

of gene transcription [2, 3, 25, 26]. However, it is still subject to discussion whether the sensing of oxygen supply and its transduction into adequate EPO synthesis are performed extrarenally or in the kidney itself [1]. A strong argument for an oxygen-sensing function of the kidney would be the demonstration that the isolated kidney is capable of producing EPO in an oxygen-dependent manner, as is known to occur in the intact organism. In fact, it has been reported that isolated perfused kidneys release more EPO when the oxygen supply is lowered [7, 9, 16, 28]. In those studies the amount of EPO released from isolated kidneys was determined indirectly either by measuring EPO dependent ^{59}Fe incorporation into red blood cells [7, 9, 28] or by determination of the proliferative activity of the erythron [16]. Moreover, those experiments were partly performed in kidneys isolated from hypoxic animals, a fact that makes it difficult to distinguish whether EPO formation had been really induced or had merely been modulated during ex vivo perfusion [7]. A major argument against an essential “oxygen sensing” by the kidney is that a severe reduction of renal oxygen supply by selective reduction of renal perfusion only causes a slight increase of EPO formation while the same decrease of oxygen supply by a reduction of the systemic oxygen transport capacity leads to an exponentially increasing EPO production [20].

In view of these conflicting findings it seemed reasonable to examine whether EPO production can be reproducibly induced by oxygen depletion in isolated perfused kidneys, taken from normoxic animals. To this end isolated kidneys were perfused at constant pressure in a recirculatory system containing red cells. To improve the functional conservation of the preparation the perfusate was continuously dialysed during the experiments. The amount of EPO released into the perfusion medium was determined by using a sensitive and specific radioimmunoassay [5].

If EPO formation could really be induced in isolated perfused kidneys taken from normoxic animals by oxygen depletion, then this model could be used to investigate the principles of the signal transduction that link EPO

production to oxygen delivery. Even though the oxygen-sensing mechanism underlying EPO formation remains rather elusive at the moment, it is conceivable that EPO synthesis might be controlled by intracellular release of second-messenger molecules. Therefore, as a first approach, we have examined the effects of the classic second messengers such as cyclic AMP, cyclic GMP and calcium/calmodulin on EPO production by the isolated perfused rat kidney.

Materials and methods

Male SIV strain rats (250–350 g body weight), which had free access to commercial chow and tap water before the experiments, were used throughout. According to the technique described by Schurek and Alt [24], kidney perfusion was performed as follows: rats were anaesthetized by intraperitoneal injection of 150 mg/kg 5-ethyl-5 (1'-methylpropyl)-2-thiobarbituric acid (Inactin, Byk Gulden, Konstanz, FRG). During preparation, respiration was maintained using a Harvard respirator (75 strokes/min, stroke volume 2 ml, 25% O₂). Following abdominal midline incision, the right ureter was cannulated with a polypropylene tube (PP-10) connected to a larger polyethylene catheter (PE-50). After intravenous heparin injection (2 U/g body weight Liquemin, Roche, Basel, Switzerland) via a catheter in the jugular vein, the kidney was placed in a metal chamber fixed at a micropuncture table. The arterial branches of the abdominal aorta were ligated and a double-barreled cannula was inserted into the aorta and placed at the origin of the renal artery. To avoid renal ischaemia, perfusion was started in situ with an initial flow rate of 8 ml/min. The kidney was isolated from the animal and, about 10 min later, perfusion at constant pressure was established. The effective perfusion pressure was monitored through the inner part of the cannula (Statham transducer P10EZ) and held constant at 100 mmHg by means of a feedback-regulated peristaltic pump. The perfusion medium consisted of a Krebs-Henseleit physiological saline containing all physiological amino acids in concentrations between 0.2 mM and 2.0 mM, 8.7 mM glucose, 0.3 mM pyruvate, 2.0 mM L-lactate, 1.0 mM 2-oxoglutarate, 1.0 mM L-malate and 6.0 mM urea. Thyroid hormone (150 ng/100 ml), [Lys⁸] vasopressin (1 mU/100 ml) and polyfructosan (1 g/l) were also added. Ampicillin and flucloxacillin (3 mg/100 ml each) were used as antibiotics. Moreover, the perfusate contained 2 g/100 ml bovine serum albumin and a 9%–11% fraction of three times freshly washed human erythrocytes. An in-line filter (8 µm pore size) was used during the preperfusion period, before erythrocytes were added, to remove small particles from the circulation. The perfusate, which was thermostated to 37 °C, was taken from a reservoir (200–220 ml). The experiments were performed in a recirculatory perfusion system. To this end the renal vein was catheterized with a metal cannula and the venous effluent was drained back into the reservoir. To improve the functional conservation of the preparation the perfusion medium was continuously dialysed against a 20-fold volume of protein-free Krebs-Henseleit solution. Oxygen delivery to the kidney was adjusted by equilibrating the dialysate with gas mixtures containing various amounts of oxygen. Oxygen partial pressure was measured in the venous outflow or in an arterial bypass using an in-line Clark type O₂-electrode (Eschweiler, Kiel, FRG). Before and after each experiment, the O₂ electrode was calibrated with nitrogen and 100% oxygen, respectively. The perfusion flow rate was obtained from the pump revolutions. Perfusion pressure, flow rate, and arterial and venous PO₂ in the perfusate were continuously monitored by a potentiometric recorder (Kipp & Zonen, Delft, Netherlands). EPO was assayed in aliquots drawn from the perfusion medium at 30-min intervals. To obtain steady-state conditions the tested drugs, dissolved in freshly prepared perfusion medium, were continuously infused into the arterial limb of the circulation directly in front of the kidney. Addition of the compounds was normally started about 30 min after the onset of perfusion and maintained for a 1-h period. A flow-proportional infusion rate of 2% of the perfusion flow was adjusted by using a peristaltic pump (2132 Microperpex, LKB, Bomm, Sweden).

Analytical methods

Determination of EPO. Before assay for EPO, perfusate samples were concentrated 5 to 7-fold by ultrafiltration through low-absorption YM membranes with a 30-kDa molecular mass cutoff and a measured recovery for ¹²⁵I-EPO of approximately 90% (Centricon microconcentrators, Amicon, USA). EPO was measured by radioimmunoassay as described [5] using a rabbit antiserum raised against recombinant human EPO and iodinated recombinant EPO (Amersham Lab., England) as tracer. A serum pool obtained from hypoxic rats was used as standard after previous determination of the EPO content by in vivo bioassay [5].

Determination of sodium and potassium. Sodium and potassium were analysed by flame photometry (Instrumentation Laboratory 943).

Determination of glomerular filtration rate. The glomerular filtration rate was obtained from polyfructosan (Laevasan, Linz, Austria) clearance. Polyfructosan was measured after acid hydrolysis by a phosphohexose isomerase reaction [23].

Calculation of oxygen consumption. Oxygen consumption of the kidneys was calculated from the arterio-venous PO₂ difference of physically dissolved and haemoglobin-bound oxygen and the perfusion flow rate. Concentrations of dissolved oxygen were calculated from the arterial and venous PO₂ values using the absorption coefficient for oxygen ($\alpha_{O_2} = 0.0227$) in physiological saline at 37 °C. For determination of haemoglobin-bound oxygen an oxygen-binding curve was made for every perfusate by tonometry (Instrumentation Laboratory 237). Haemoglobin concentrations were measured spectrophotometrically (CO-Oximeter, Instrumentation Laboratory 282). Arterio-venous differences of haemoglobin-bound oxygen were then calculated from the oxygen-binding curves, the haemoglobin concentrations and the respective arterial and venous PO₂ values.

Materials. Pyruvate and the test kit for polyfructosan determination were obtained from Boehringer, Mannheim, FRG. Ampicillin and flucloxacillin were from Beecham, Bern, Switzerland. Glutamate, urea, and 2-oxoglutarate were provided by Merck, Darmstadt, FRG. L-Malic acid and L-lactate as sodium salts were obtained from Serva, Heidelberg, FRG. Bovine serum albumin, cyclic 8-bromo-GMP and N-(6-aminoethyl)-5-chloro-1-naphthalenesulphonamide (W-7) were from Sigma International. Thyroid hormone was supplied by Henning, Berlin, and L-amino acids by Braun/Melsungen, FRG (Aminoplasmal paed. free of carbon hydrates). Polyfructosan was purchased from Laevasan, Linz, Austria, and [Lys⁸]vasopressin from Sandoz, Basel, Switzerland. Forskolin was obtained from Calbiochem, Luzern, Switzerland, and calmidazolium from Janssen, Olen, Belgium.

Statistics. Student's *t*-test was used for comparison of groups and analysis of variance to determine levels of significance. *P* < 0.05 was considered significant.

Results

EPO release from kidneys perfused at PO₂ values of 500 mmHg and 120 mmHg is shown in Fig. 1a. At 500 mmHg the amount of EPO released by the kidneys rose almost linearly during the first 3 h of perfusion and reached a constant level further on. For this condition the EPO production rate was calculated to be 0.17 ± 0.02 U h⁻¹ g kidney⁻¹ (mean \pm SEM, *n* = 5). When perfused at an oxygen pressure of 120 mmHg, EPO release significantly increased 60–90 min after the onset of perfusion (*P* < 0.05). After 3.5 h of perfusion the amount of EPO present in the perfusate remained constant. For the phase of linear increase (between 90 min and 150 min) the EPO production rate was calculated to be 1.2 ± 0.1 U h⁻¹ g

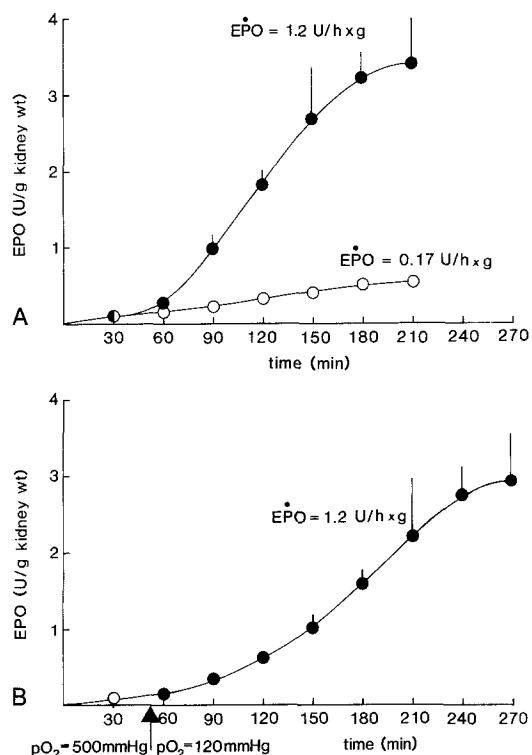


Fig. 1. Time-dependent erythropoietin (EPO) production by isolated rat kidneys (A) perfused at arterial PO_2 values of 500 mmHg (\circ) and 120 mmHg (\bullet) and (B) perfused first at 500 mmHg (\circ) and subsequently at 120 mmHg (\bullet). A Increase of erythropoietin release from kidneys perfused at PO_2 values of 120 mmHg was statistically significant from 60 min after the onset of perfusion ($P < 0.05$). EPO formation rates were calculated for the phase of linear increase. Values are means \pm SEM, $n = 5$. B Arterial PO_2 was lowered to 120 mmHg 50 min after the onset of perfusion (arrow). Note the time lag of 60–90 min between decrease of oxygen supply and significant increase of EPO release ($P < 0.05$). EPO formation rate (mean \pm SEM, $n = 5$) was calculated for the phase of linear increase

kidney⁻¹ (mean \pm SEM, $n = 5$). To test the possibility that the low EPO formation rate of 0.17 ± 0.02 U h⁻¹ g kidney⁻¹ at high oxygen pressure might not result from oxygen-dependent regulation of EPO synthesis, but rather from a toxic effect of the high oxygen pressure on the mechanisms controlling EPO formation, kidney perfusion was started at an arterial PO_2 of 500 mmHg, which was lowered to 120 mmHg 50 min after the onset of perfusion (Fig. 1b). Again, reduction of oxygen pressure was followed by a significantly ($P < 0.05$) increased EPO formation rate of 1.2 U h⁻¹ g kidney⁻¹ with a delay of 60–90 min. In further experiments PO_2 was lowered from 500 mmHg to 300 mmHg and from 500 mmHg to 30 mmHg 50 min after the onset of perfusion. As in the experiments shown in Fig. 1b, EPO release increased 60–90 min after reduction of the renal oxygen supply. The relationship of EPO production rates to the PO_2 values in the perfusate is shown in Fig. 2. Apparently, EPO production rates and PO_2 values were negatively correlated in an almost linear fashion. The effects of the oxygen tension in the perfusate on flow rate, glomerular filtration rate, sodium reabsorption and oxygen consumption of the isolated perfused rat kidney are summa-

Table 1. Functional parameters of isolated perfused rat kidneys^a

Parameter	PO_2 (mmHg)	Value after		
		30 min	90 min	150 min
Perfusion flow rate (ml min ⁻¹ g ⁻¹)	500	11 \pm 2	12 \pm 2	11 \pm 2
	120	12 \pm 1	14 \pm 2	15 \pm 2
GFR (ml min ⁻¹ g ⁻¹)	500	1.3 \pm 0.1	1.2 \pm 0.1	1.1 \pm 0.1
	120	1.1 \pm 0.1	1.0 \pm 0.1	0.8 \pm 0.1
Na reabsorption (% of tubular load)	500	87 \pm 2	73 \pm 6	62 \pm 4
	120	82 \pm 4	65 \pm 6	56 \pm 6
QO_2 (μ mol min ⁻¹ g ⁻¹)	500	6.4 \pm 1.2	6.5 \pm 1.0	5.0 \pm 1.4
	120	6.2 \pm 0.9	4.8 \pm 0.9	3.9 \pm 0.6

^a Functional parameters of kidneys perfused at arterial PO_2 values of 500 mmHg and 120 mmHg, respectively. Values are expressed as means \pm SEM, $n = 5$; GFR, glomerular filtration rate; QO_2 , renal oxygen consumption

rized in Table 1. None of these parameters was significantly different between oxygen pressures of 500 mmHg and 120 mmHg. It should be noted that the filtration fractions were relatively low ($10.4 \pm 0.9\%$, $n = 5$) because of the high perfusate flow rates observed under our experimental conditions.

In a second set of experiments we tested whether the increase of EPO production by the isolated perfused rat kidney upon lowering the oxygen pressure could be mimicked by classic second-messenger molecules. We used forskolin (10 μ M) and cyclic 8-bromo-GMP (100 μ M) to increase cellular levels of cAMP and cGMP, respectively. Calmidazolium (1 μ M) was taken to inhibit calmodulin activity [10]. All tested drugs were continuously and flow-proportionally infused into the arterial limb of the circulation for a 45-min period starting 30 min after the onset of perfusion. As shown in Fig. 3, forskolin (10 μ M) and 8-bromo-cGMP (100 μ M) caused a slight but statistically not significant ($P > 0.05$) decrease of EPO formation rates to 0.08 ± 0.02 and 0.07 ± 0.02 U h⁻¹ g⁻¹ respectively, when perfusion was performed at a PO_2 of 500 mmHg ($n = 3$ for each condition). At the same time forskolin

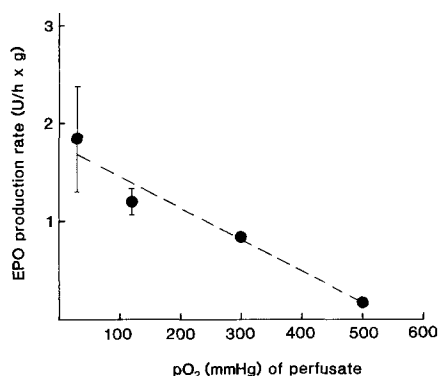


Fig. 2. Relationship between erythropoietin (EPO) production rate in the isolated perfused rat kidney and oxygen pressure in the perfusate. EPO formation and arterial PO_2 values are inversely correlated in an almost linear fashion (regression curve, $P < 0.001$). Values are means \pm SEM, $n = 5$

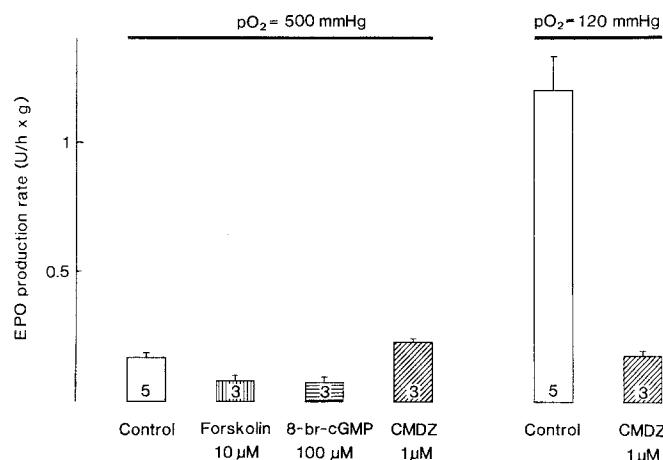


Fig. 3. Erythropoietin (EPO) production rates in the isolated perfused rat kidney obtained upon addition of forskolin (10 μ M), 8-bromo-cGMP (100 μ M), and calmidazolium (1 μ M), respectively. The tested drugs were continuously infused into the arterial limb of the perfusion circuit for a 1-h period. Infusion was normally started 30 min after the onset of perfusion. Calmidazolium (1 μ M) blunted the increase of EPO formation upon lowering arterial PO_2 from 500 mmHg to 120 mmHg ($P < 0.01$). Values are means \pm SEM; the number of experiments is indicated at the bottom of each bar

and 8-bromo-cGMP decreased renal vascular resistance (Table 2). Moreover, the drop of oxygen consumption that appeared during the course of the experiments (Table 1) was less pronounced in presence of forskolin, 8-bromo-cGMP and calmidazolium. While calmidazolium had no significant effect on EPO release at an arterial PO_2 of 500 mmHg it blunted the increase of EPO pro-

Table 2. Effect of forskolin, 8-bromo-cGMP, and calmidazolium on isolated perfused rat kidneys^a

Parameter	Value after		
	30 min	90 min	150 min
Perfusion flow rate (ml min ⁻¹ g ⁻¹)			
Forskolin (10 μ M)	13 \pm 1	16 \pm 2*	13 \pm 2
8-Bromo-cGMP (100 μ M)	15 \pm 1	22 \pm 1*	22 \pm 1*
Calmidazolium (1 μ M)	13 \pm 2	12 \pm 1	11 \pm 1
GFR (ml min ⁻¹ g ⁻¹)			
Forskolin (10 μ M)	1.3 \pm 0.1	1.0 \pm 0.1	0.9 \pm 0.1
8-Bromo-cGMP (100 μ M)	1.5 \pm 0.1	1.3 \pm 0.1	1.0 \pm 0.1
Calmidazolium (1 μ M)	1.1 \pm 0.2	1.2 \pm 0.1	1.4 \pm 0.1
Na reabsorption (% of tubular load)			
Forskolin (10 μ M)	73 \pm 1	76 \pm 3	68 \pm 1
8-Bromo-cGMP (100 μ M)	86 \pm 3	77 \pm 1	67 \pm 6
Calmidazolium (1 μ M)	90 \pm 2	77 \pm 7	67 \pm 12
QO_2 (μ mol min ⁻¹ g ⁻¹)			
Forskolin (10 μ M)	6.5 \pm 0.1	6.6 \pm 0.1	6.1 \pm 0.1
8-Bromo-cGMP (100 μ M)	6.8 \pm 0.3	6.2 \pm 0.6	5.7 \pm 0.2
Calmidazolium (1 μ M)	7.2 \pm 0.3	6.7 \pm 0.1	6.1 \pm 0.3

^a Kidneys were perfused at PO_2 values of 500 mmHg. The tested drugs were continuously infused into the arterial limb of the circulation for a 45-min period starting 30 min after the onset of perfusion. Values are means \pm SEM, $n = 3$ for each condition

* Significantly different from control ($P < 0.05$)

Table 3. Effect of calmidazolium on functional parameters of isolated rat kidneys perfused at PO_2 values of 120 mmHg

Parameter	Value after		
	30 min	90 min	150 min
Perfusion flow rate (ml min ⁻¹ g ⁻¹)			
Calmidazolium	12 \pm 1	12 \pm 1	12 \pm 1
Control	10 \pm 2	11 \pm 2	13 \pm 3
GFR (ml min ⁻¹ g ⁻¹)			
Calmidazolium	1.3 \pm 0.2	1.1 \pm 0.1	1.0 \pm 0.2
Control	1.2 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.1
Na reabsorption (% of tubular load)			
Calmidazolium	86 \pm 4	69 \pm 4	57 \pm 4
Control	93 \pm 1	76 \pm 4	59 \pm 3
QO_2 (μ mol min ⁻¹ g ⁻¹)			
Calmidazolium	6.1 \pm 0.4	3.8 \pm 0.3	3.3 \pm 0.4
Control	6.2 \pm 1.1	4.0 \pm 0.7	3.9 \pm 0.9

^a Functional parameters of isolated rat kidneys. At 45 min after the onset of perfusion, arterial PO_2 was lowered from 500 mmHg to 120 mmHg. Calmidazolium (1 μ M) was continuously added for a 45-min period starting 30 min after the onset of perfusion. Calmidazolium was omitted in controls. Values are means \pm SEM, $n = 3$

duction when the PO_2 was lowered from 500 to 120 mmHg, without altering other functional parameters (Fig. 3, Table 3), in particular the oxygen consumption rates. W-7 (10 μ M), a further putative calmodulin inhibitor, which structurally differs from calmidazolium [11], also prevented the rise of EPO formation at PO_2 values of 120 mmHg (EPO production rate: 0.24 ± 0.07 U h⁻¹ g⁻¹; $n = 4$). When forskolin (10 μ M) and 8-bromo-cGMP (100 μ M) were applied at a PO_2 of 120 mmHg, EPO formation rates remained unchanged (1.1 ± 0.1 and 1.0 ± 0.2 U h⁻¹ g⁻¹, respectively; $n = 3$ for each condition). At the same time, forskolin (10 μ M) and 8-bromo-cGMP (100 μ M) increased renal perfusate flow by $52 \pm 3\%$ and $43 \pm 5\%$ of control values without affecting other functional parameters, such as oxygen consumption.

Discussion

Our findings agree with and extend previous observations that the isolated perfused kidney releases erythropoietin (EPO) in an oxygen-dependent manner [7, 9, 16, 28]. Our data show that hypoxia-induced stimulation of EPO release is a phenomenon that is not just restricted to isolated perfused kidneys taken from hypoxic animals [7] but is also observed with kidneys taken from normoxic rats [9, 16, 28]. Since the normoxic rat kidney contains no stores for EPO [13] one can infer that the isolated perfused rat kidney is capable of de novo synthesis of EPO. EPO formation by the isolated perfused rat kidney moreover displays some characteristics that are also observed in vivo. First, it is inversely proportional to renal oxygen supply (Fig. 2, [17]). Secondly, the time lag of 60–90 min between decrease of oxygen supply and increase of EPO release (Fig. 1) is similar to the period after which serum

EPO levels start to increase upon hypoxia in vivo [17]. And finally, the maximal rate of EPO production, which is estimated to be around $2 \text{ U h}^{-1} \text{ g kidney}^{-1}$ in vivo [17], is comparable to the values calculated for the isolated perfused kidney. What differs in vivo is that the isolated perfused rat kidney stops producing EPO after 3 h of perfusion. We think that this phenomenon results from a functional exhaustion of the preparation, because other parameters, such as the sodium reabsorption rate and thereby the renal oxygen consumption, steadily decrease during the time of perfusion (Table 1). This functional deterioration is known to be a characteristic feature of kidneys perfused in vitro. Nevertheless, the continuous decrease of oxygen consumption, as particularly observed during perfusion at low PO_2 values, was not rate-limiting for the hypoxia-induced elaboration of EPO by the isolated perfused rat kidney. Thus, our findings suggest that the essentials of the oxygen sensor controlling EPO formation are predominantly located in the kidney itself.

These data seem to be in contrast to the observation that selective reduction of oxygen supply to the kidneys by clamping of the renal arteries only causes a slight increase of the EPO formation rate, whereas lowering the systemic oxygen-transport capacity results in an exponential rise of serum EPO levels [20]. It should be remembered, however, that reduction of renal perfusion flow not only causes a reduction of renal oxygen delivery but also decreases the tubular sodium load and thereby locally alters renal oxygen consumption [4].

The signal transduction process that links EPO formation to the oxygen supply has not yet been elucidated, and it appears as if the isolated perfused rat kidney could be a suitable model to study this process. Recently, it was reported that adenosine causes a twofold stimulation of EPO release from the isolated perfused rat kidney [21]. Since intrarenal levels of adenosine increase during insufficient oxygen supply, it was suggested that adenosine could be a messenger molecule between oxygen supply and EPO production [8, 19, 22]. Cyclic AMP, cyclic GMP and calcium/calmodulin have also been suggested to have a role in this signal transduction process [8]. To test for a possible involvement of cyclic nucleotides in the signal transduction process, we used forskolin and 8-bromo-cGMP to increase cellular levels of cAMP and cGMP, respectively. Both drugs significantly decreased renal vascular resistance, but at the same concentrations neither forskolin nor 8-bromo-cGMP stimulated EPO production in kidneys perfused at PO_2 values of 500 mmHg. Calmidazolium, a putative calmodulin antagonist [10], also had no stimulatory effect on EPO formation at high PO_2 values.

It has been demonstrated that increasing cellular levels of cyclic AMP with forskolin had no effect on EPO formation in human hepatoblastoma (Hep G2) cells grown under normoxic conditions [27]. At the same time, however, forskolin further enhanced the stimulatory effect of hypoxia on EPO secretion from Hep G2 cells exposed to an atmosphere of 1% oxygen [27]. Therefore, in the present study, we have also examined the effects of forskolin and 8-bromo-cGMP on EPO production in iso-

lated kidneys perfused under hypoxic conditions. Our finding that both drugs had no effect, in particular no additional stimulatory effect, on EPO production rates in isolated rat kidneys perfused at PO_2 values of 120 mmHg could indicate that the signal transduction mechanisms for EPO formation might not be principally the same in the human hepatoblastoma cell line Hep G2 as in the intact kidney.

Even more interesting might be our observation that the calmodulin antagonist calmidazolium blunted the increase of EPO formation when the oxygen pressure was lowered in the perfusate. This effect of calmidazolium was confirmed with W-7, another putative calmodulin inhibitor [11], which structurally differs from calmidazolium. Recently, evidence has been provided for a link between the regulation of EPO production and proximal-tubular energy consumption [6]. It might be conceivable, therefore, that calmodulin antagonists exert their effects by lowering the activity of the proximal tubular work. However, as compared to controls, calmidazolium and W-7 had no obvious side-effects on renal functional parameters, particularly not on sodium reabsorption and oxygen consumption. Moreover, since all of these drugs were constantly infused into the kidneys and at the same time continuously removed from the circulation by dialysis, it appears rather unlikely that the inhibitory action of calmidazolium and W-7 on hypoxia-induced EPO formation was a toxic effect due to accumulation of the compounds. It seems possible, therefore, that a calmodulin-activated reaction is somehow involved in the oxygen-dependent EPO production. From the observation that calmidazolium and W-7 both blunted the increase of EPO formation induced by oxygen depletion whereas basal EPO production (at a PO_2 of 500 mmHg) remained nearly unaffected, one might infer that these drugs affect the oxygen-sensing mechanism rather than the synthesis and processing of EPO. The identification of this reaction step remains a task for future research.

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