Acute Erythropoietin Infusion Increases Rat Glomerular Filtration Rate by Partly Stimulating Intrarenal Nitric Oxide Production

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(Received December 8, 2003; Accepted May 6, 2004)

Currently, erythropoietin (EPO) treatment in anemic patients is getting more interest, but in long term, development of hypertension is common problem in EPO treated patients. Since it isn't yet known whether EPO affects directly vascular and/or kidney functions, we aimed to study effects of acute EPO treatment on renal tissue nitrite level, and cardiovascular function and glomerular filtration rate (GFR). Experiments were done under 4 groups. Group I: Sham operated control, group II: 150 IU/kg EPO, group III: 50 mg/kg Nw-nitro-L-arginine methyl ester (L-NAME), group IV: L-NAME+EPO. Femoral artery, vein and bladder were catheterized under anesthesia. After stabilization and first 45 min basal control period, drugs were given i.v. bolus infusion according to the group protocols, then 2 × 45 min clearance periods were followed. EPO infusion increased renal tissue nitrite activity causing GFR increase without any influence on systemic blood pressure. L-NAME alone or together with EPO significantly raised the systemic blood pressure with a partial increase in GFR, but L-NAME treatment significantly reduced tissue nitrite level. The present study for the first time suggests that exogenous EPO treatment increases rat GFR and renal tissue nitrite level without affecting systemic blood pressure.

Key words ——— erythropoietin, nitric oxide, renal tissue nitrite level, blood pressure, glomerular filtration rate, Nw-nitro-L-arginine methyl ester

INTRODUCTION

Erythropoietin (EPO) is the principal regulator of erythropoiesis. A relative deficiency of EPO is the main cause of the anemia in end stage renal disease (ESRD). In addition hemolysis, short life span of erythrocytes or blood losses from several sources including hemodialysis worsens the anemia in ESRD patients. Human recombinant EPO (rHuEPO) has gained a central role in the treatment of the anemia in ESRD, but use of EPO is associated with a number of adverse effects.[1,2] The common and potentially most serious side effect is development or aggravation of hypertension which has been partially explained with an increase in blood viscosity, a diminished hypoxic vasodilatation or an enhanced cardiac output due to a better myocardial oxygenation.[3,4] However several studies indicate that amelioration of anemia is not the primary mechanism of EPO induced hypertension. A lot of theories have been postulated to explain this problem. Some of these theories are elevation of basal cytosolic Ca^{+2} level in vascular smooth muscle cells, increase in vasopressor response to norepinephrine, increased endothelin-1 (ET-1) production, increase in expression of renin mRNA and angiotensinogen mRNA in kidney and aorta, increased release of vasoconstrictive prostaglandin F2α, thromboxane B2 and decrease in release of vasodilatory prostaglandin and prostacyclin, direct vasopressor effects and vascular remodeling with increasing mitogenic activity in the smooth muscle cells.[3,5–9] Nevertheless, the results of these studies are quite different from each other and conflicting. Furthermore, another subject of this discussion is the onset time of hypertension.

Kidney is a very important organ for development and control of hypertension. Studies concerning the effect of EPO on kidney are limited and not enough to explain acute and chronic effects of EPO

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treatment on renal functions. In this study we tried to explore the acute effects of EPO infusion on the cardio-renal system and the role of nitric oxide in the rat kidney.

MATERIALS AND METHODS

Thirty six ($n = 9$ for each group) Sprague Dawley rats, weighing 250–300 g were used in the present study. The rats were provided by Osmangazi University Experimental and Medical Research Center and the experimental protocol was approved by the university ethical committee of animal studies.

Experiments were done in four groups. 1: Sham operated control, 2: 150 IU/kg rHuEPO (Eprex, SantaFarma), 3: 50 mg/kg Nw-nitro-L-arginine methyl ester (L-NAME) (Sigma, St. Louis, U.S.A.) and 4: EPO+L-NAME i.v. infused group.

The animals were anesthetized with thiopental (40 mg/kg, i.p.) and placed prone position on a warmed surgical plate to maintain body temperature at 37 ± 0.5°C throughout the procedure. Surgical area was disinfected with betadine solution. The femoral artery and vein were catheterized with PE50 cannula and femoral artery was used to monitorize the direct blood pressure parameters as intraarterial systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP) and heart rate (HR) *via* a pressure transducer connected to a data acquisition system (Biopac-USA, Santa Barbara, U.S.A.). The femoral vein was used for infusion of drugs and fluid supply. After an abdominal incision, urinary bladder was catheterized with PE100 cannula for timed urine collection. Continuous infusion of 3% creatinine in saline at a rate of 25 μl/min performed throughout the experiments. The experiments were done in three consecutive 45 min durations. After 30 min stabilization period following surgical procedures, the first 45 min clearance time was considered as basal control. The second and the third 45 min periods were drug exposure times of the groups. At the end of each period, 0.2 ml blood was withdrawn, the timed urine samples were collected for the clearance measurements and blood pressure parameters were recorded. The drugs or the same volume of SF were given by i.v. injection at the beginning of the second period according to the group protocol. The blood samples were centrifuged at 4000 rpm, 4°C for 10 min. The serum were removed and used for measurement of creatinine clearance which was used for estimation of glomerular filtration rate (GFR).

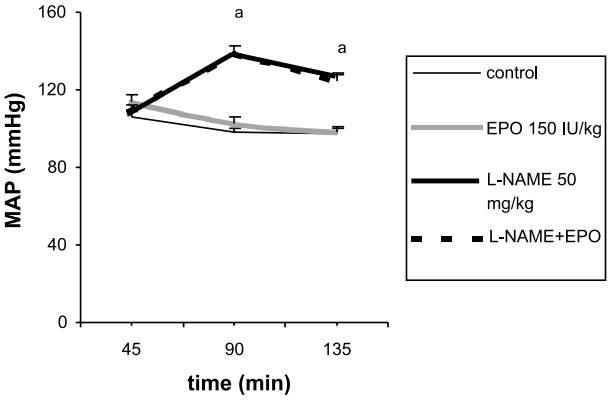

Fig. 1. Time Dependent Changes in MAP

Data were given as mean ± standart errors (S.E.). (a) Represents the difference between the control and L-NAME, L-NAME+EPO groups ($p < 0.001$).

The stable nitric oxide (NO) end product, nitrite was quantitated in plasma and renal tissue. Nitrite level was measured by Griess reaction. Tissue samples were homogenized in 0.1 M PBS (pH = 7.4) and centrifuged. Supernatants were diluted and used for nitrite measurement.[10] The same procedure was applied to the plasma samples.

One way analysis of variance (ANOVA) and Tukey analysis were applied for comparisons among the groups. Results are given as mean (standard error and $p < 0.05$ is accepted as statistically significant.

RESULTS

Acute Effects of EPO on Blood Pressure

Acute injection of 150 IU/kg EPO alone did not cause significant changes in systemic blood pressure parameters of SP, DP and HR (data not shown), but inhibition of NO synthesis with L-NAME or L-NAME+EPO infusion increased blood pressure ($p < 0.001$). Measurements of MAP obtained with bolus injection of EPO and L-NAME are shown in Fig. 1.

Effects of EPO Infusion on Urine Excretion and GFR

L-NAME infusion resulted in prominent diuresis which increased with time ($p < 0.001$). Similar increase was seen in L-NAME+EPO group (Fig. 2). EPO infusion also caused increase in urine volume but it was not as much as in L-NAME group ($p < 0.05$). Pressure diuresis was prominent in L-NAME treated groups. EPO increased rat urine flow

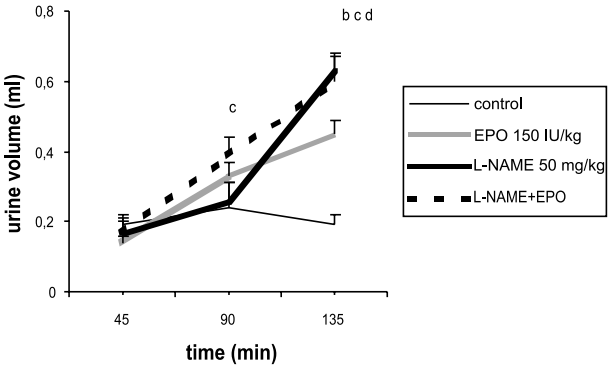

Fig. 2. Urine Volume Changes with Time

Data were given as mean ± S.E. (b) Represents the difference between the control and EPO groups ($p < 0.05$). (c) Represents the difference between the control and L-NAME+EPO groups ($p < 0.001$). (d) Represents the difference between the control and L-NAME groups ($p < 0.001$).

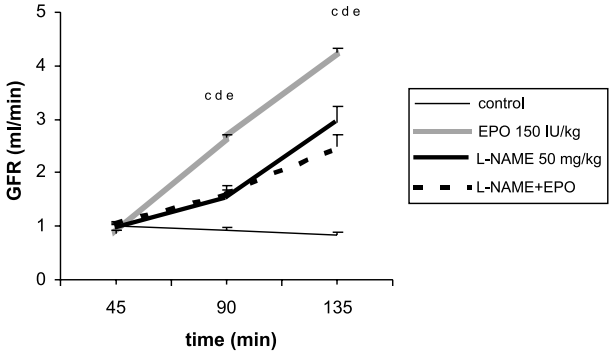

Fig. 3. GFR Changes with Time

Data were given as mean ± S.E. (e) Represents the difference between the EPO and control, L-NAME, L-NAME+EPO groups ($p < 0.001$).

rate without affecting renal perfusion pressure.

GFR was increased significantly after EPO injection alone without changing renal perfusion pressure. L-NAME or L-NAME+EPO infusions were also increased GFR (Fig. 3) with a significant elevation of renal perfusion pressure ($p < 0.001$).

Renal Tissue Nitrite Levels

There was no difference between plasma nitrite levels of the groups (Data not shown). EPO infusion increased renal tissue nitrite level when compared to the control ($p < 0.05$). In the L-NAME or L-NAME+EPO infused groups we found decreased nitrite production in renal tissue (Table 1).

Table 1. Renal Tissue Nitrite Levels

GROUP	Tissue nitrite levels (μM)
CONTROL	339 ± 8
EPO 150 IU/kg	381 ± 13[a)]
L-NAME 50 mg/kg	312 ± 9[b)]
L-NAME+EPO	325 ± 11

Data were given as mean ± S.E. *a*) represents the difference between the control and EPO groups ($p < 0.05$). *b*) represents the difference between the control and L-NAME groups ($p < 0.05$).

DISCUSSION

Blood pressure regulation under physiologic and pathophysiologic conditions has been attracting many researchers for many years, because of severe effects of blood pressure disorders including hypertension on human health. Since development of hypertension is common in EPO treated uremic patients we studied the acute effect of EPO treatment (150 IU/kg) on cardio-renal functions of rats rather than its hematopoietic activity.

The present study demonstrated that the acute EPO infusion increased urine volume and GFR without changing blood pressure in rats. It is well known that nitric oxide plays several crucial roles in the regulation of blood pressure and renal function. Thus, we also measured the nitrite level, one of the metabolites of nitric oxide, in renal tissue and plasma with a spectrophotometric method. EPO treatment significantly increased the renal tissue nitrite level but not plasma nitrite implying that EPO especially affects the intrarenal nitric oxide pathway. This result may be a direct vasodilatory effect of EPO induced secretion of NO from renal vasculature or might be a response of renal tissue to vasoconstrictor effect of EPO upon renal vessels. Heindereich *et al*. reported that EPO exerted a direct vasopressor effect on proximal resistance vessels of the kidney of normal rats and this effect started from 10 IU/ml, reaching a maximum response at 200 IU/ml EPO.[11] Similarly, Vaziri *et al*. showed a significant contraction of rat caudal artery rings with EPO at a 200 IU/ml concentration.[7] Kang *et al*. found that plasma levels of proET-1 and ET-1 increased after a single intravenous administration of rHuEPO which can be associated with an acute elevation of blood pressure.[12] In the present study EPO induced vasoconstriction of renal vessels might increase nitrite production causing urine volume and GFR increases. However, conflicting results were also reported by Bode-Boger *et al*. They did not find a significant

contraction response to EPO on rabbit aorta rings.[13] Moreover, Banerjee *et al*. found that in 24 hr exposure of human umbilical vein endothelial cell in culture to EPO caused a threefold increase in nitric oxide synthase (NOS) transcription.[14] It seems like that responses of various vascular structures to EPO might also be different. In agreement Wu *et al*. showed that EPO treatment modified the responses and responsiveness to a number of vasoconstrictor and vasodilator compounds in renal interlobular arteries and subcutaneous arteries from patients with renal disease.[15] Del Castillo *et al*. have reported an increased urinary excretion of NO^{-2} and NO^{-3} in rats treated with rHuEPO. They concluded that decreased activity of endogenous nitric oxide system does not underlie the pressor effect of rHuEPO in normal animals.[16] Similarly, Miglion *et al*. measured the urinary NO metabolites ($NO^{-2} + NO^{-3}$) and indicated that the endogenous NO system activity is enhanced in rHuEPO induced hypertension in rats with normal renal function and a resistance to NO was not developed in renal circulation.[9] In our study infusion of EPO also resulted in an increased production of nitrite in renal tissues of normal rats and this effect blocked by systemic administration of NOS synthase inhibitor, L-NAME.

On the other hand, NO might be released under hypoxia, as well. Some studies indicated that acute hypoxia upregulates NOS gene expression in different organ systems of the rat such as kidney, lung and heart. Nitric oxide is a potent modulator of mitochondrial respiration, ATP synthesis and K (ATP) channel activity. During hypoxia, mitochondrial NOS (mtNOS), which is an active eNOS-like isoform, involves in altered mitochondrial regulation. NOS I and NOS III mRNA levels were also found upregulated by hypoxia in heart, kidney and lung.[17] By increasing synthesis of NO in specific tissues assures the blood flow to tissues *via* vasodilatation.

Besides direct vasodilatory effect, NO has an inhibitory effect on local hypoxia induced factor (HIF-I) activity. Increased activity of NO may be also important for regulation of HIF-I activity. HIF-I stimulates glucose transport to cells. Under hypoxic conditions, energy requirement of cells mainly supplied by glycolysis rather than oxidative phosphorylation.[18] This is very important for tissues to adapt to hypoxia.

NO is regulator of oxygen usage in kidney and important for arrangement of chemical work of kidney. It is shown that NO releasing substances reduced consumption of ATP for each Na absorption from proximal tubules. NO inhibits cytochrome c activity of mitochondria in heart and skeletal muscle. Na-K ATPase activity is very important for kidney and it consumes too much energy. The energy that used for sodium reabsorption and work are directly related with O_2 consumption of kidney and determines the O_2 needs of the body also. Whereas NO releasing substances like bradykinin decreases the medullar and cortical O_2 dependency, NOS blocker, L-NAME increases ATP consumption per Na^+ absorbed.[11] In normal body, exogenous EPO infusion will be recognized as an alarm signal for the body. Because EPO is secreted a direct response to hypoxia, body will try to decrease O_2 consumption. All of these results from cell culture experiments, different effects on different vessel types and effects of NO on cell metabolism suggest that increased production of NO after EPO infusion may not be only response to vasoconstrictor effect of EPO and it may be a mediator of EPO in kidney during hypoxia.

In spite of EPO induced increases in renal nitrite level and GFR, systemic blood pressure was not affected in the present study. NO inhibition by L-NAME increased the rat blood pressure and pressure induced diuresis. L-NAME+EPO infusion did not cause further increases in the systemic blood pressure but blocked EPO induced GFR increases. Therefore, we suggest that EPO increase in renal tissue would be interpreted as hypoxic emergency and NOS activity might be increased. Increase in NO production in renal tissue may cause urine flow rate increase and increase was more prominent in GFR. GFR increase was either *via* vasodilation of renal vasculature and/or especially shifting main ATP production from renal oxidative phosphorylation to glycolytic pathway to slow down the metabolism which in turn decreases Na/K ATPase activity causing natriuresis and diuresis. These events started by EPO may be specific for kidney tissue. Since increase in GFR mostly related with NO and partly can be blocked by L-NAME infusion, but increase of GFR was more than increase in urine volume. NO may take part in mostly tubular effects of erythropoietin rather than glomerular effects of EPO. Other factors apart from NO could not be excluded, as well.

In conclusion, acute *in vivo* EPO infusion increases GFR and urine volume in renal tissue with-

out affecting blood pressure and heart rate in rats. NO is partly responsible of EPO induced changes in rat renal functions.

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