Recombinant Human Erythropoietin Stimulates Tubular Reabsorption of Sodium in Anesthetized Rabbits

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To determine whether recombinant human erythropoietin (rHuEPO) exerts a direct vasoconstrictive effect on renal arteries or affects renal function, we measured renal hemodynamics and renal function during a 30-min intrarenal infusion of rHuEPO in anesthetized rabbits without renal failure. Intrarenal infusion of rHuEPO at a rate of 100 U/min did not alter mean arterial pressure, renal blood flow, or renal vascular resistance, as compared with controls treated with vehicle. There were no significant rHuEPO-associated changes in glomerular filtration rate, filtration fraction, or arterial hematocrit. However, urine volume, urinary excretion of sodium and potassium, and fractional sodium excretion were significantly reduced by intrarenal infusion of rHuEPO. These observations indicate that rHuEPO has no direct effects on mean arterial pressure or renal hemodynamics, but that it stimulates net tubular sodium reabsorption, and reduces urine volume and urinary excretion of sodium and potassium in anesthetized rabbits without renal failure. (Hypertens Res 1995; 18: 203-207)

Key Words: chronic renal failure, anemia, recombinant human erythropoietin (rHuEPO), hypertension, renal hemodynamics

Since it was molecularly cloned and a biologically active form was expressed in mammalian cells (1, 2), recombinant human erythropoietin (rHuEPO) has been produced and used clinically to treat anemia associated with chronic renal failure (3-5). Its use is associated with a sustained, dose-dependent rise in hematocrit that effectively abolishes the symptoms of anemia. The main side effect limiting use of rHuEPO in dialysis and pre-dialysis patients is the development or the aggravation of hypertension, which has been attributed to an increase in blood viscosity elicted by the rise in hematocrit (4, 6) or to vasoconstriction induced by the improvement of hypoxia (3, 7). Recently, Heidenreich et al. (8) have reported that rHuEPO has a direct vasopressor effect on renal proximal resistance vessels with internal diameters between 200 and 250 μm that were obtained from Wistar-Kyoto rats without renal failure. They suggested that this direct vasopressor effect may contribute to the development of hypertension in rHuEPO-treated dialysis patients.

The present study was designed to determine whether rHuEPO has direct effects on mean arterial pressure or renal hemodynamics in in vivo anesthetized rabbits without renal failure. The effects of rHuEPO on urine volume and urinary excretion of sodium and potassium were examined and compared with the results in a vehicle-treated control group.

Methods
Twelve female albino rabbits weighing 2.7 to 3.5 kg were maintained on standard rabbit chow, which provided 9.1 meq sodium/100 g and 63.4 meq potassium/100 g, and were allowed free access to water. Experiments were performed after rabbits fasted overnight (20 h) and water was allowed ad libitum. The rabbits were anesthetized with urethan (450 mg/kg iv) and a-chloralose (45 mg/kg iv). Tracheostomy was then performed, and the trachea was intubated to allow spontaneous breathing. A catheter (PE-50) was inserted into the inferior vena cava through the left femoral vein for infusion of 5% dextrose solution. Another polyethylene catheter (PE-60) was inserted through the left femoral artery into the abdominal aorta adjacent to the renal arteries for monitoring of arterial pressure and collection of arterial blood samples. The left renal artery was then exposed through a left retroperitoneal flank incision and gently isolated. A noncanulating electromagnetic flow probe (2.0 mm,
Nihon Kohden, Tokyo, Japan) connected to an electromagnetic flowmeter (MF-27 Nihon Kohden) was placed around the renal artery to measure renal blood flow. Arterial pressure was monitored with a pressure transducer and amplifier (Biophysiograph, 180 system, SAN-EI, Tokyo, Japan). Arterial pressure and renal blood flow measurements were recorded on a pen oscillograph. The left ureter was then cannulated with tubing for collection of urine. A curved needle (27G) attached to a polyethylene catheter (PE-50) was then placed into the left renal artery proximal to the electromagnetic flow probe for infusion of rHuEPO or vehicle. Recombinant HuEPO was provided by Sankyo (Epoetin Alfa, Sankyo Co., Ltd., Tokyo, Japan). A priming infusion of a 5% dextrose solution was administered intravenously in a volume of up to 2% of body weight. This was followed by a continuous infusion of 5% dextrose solution at a rate of 0.411 ml/min and after the equilibrium period. During the equilibrium period, a priming dose of 50 mg/kg of creatinine in 4 ml of 5% dextrose solution was administered via the left femoral vein catheter. This was followed by a prolonged infusion of 0.5 mg/kg/min of creatinine in 0.411 ml/min of 5% dextrose solution, which was continued for the duration of the experiment to permit estimation of the glomerular filtration rate. Isotonic saline was infused via a catheter into the left renal artery at a rate of 0.0656 ml/min for the duration of the experiment. Experimental protocols were initiated at least 60 min after the start of the creatinine infusion to permit stabilization of arterial blood pressure and renal blood flow.

Rabbits (n = 12) were divided into a time control group and a rHuEPO group. Measurements were obtained during three consecutive 10-min periods to serve as basal values. During each 10-min period and at the end of the third basal period, a urine sample and a 5-ml sample of arterial blood were obtained and the following variables were determined: urine volume (UV); urinary excretion of creatinine (Ucr), urea nitrogen (UUN), sodium (UNa), and potassium (UK); systemic arterial hematocrit (Hct); and plasma concentrations of creatinine (Pcr), sodium (PNa), and potassium (PK). Thereafter, in the rHuEPO group, rHuEPO was infused intrarenally at a rate of 100 U/min intravenously. The infusion rate of rHuEPO was chosen based on the results of a preliminary study. In that study, the arterial concentration of rHuEPO was 0.19 ± 0.014 U/ml (n = 6) and did not change significantly during the experiment in the time control group, while the arterial concentration of rHuEPO after a 30-min intrarenal infusion of rHuEPO at a rate of 100 U/ml increased to 27.3 ± 3.62 U/ml (n = 6), which was higher than the threshold concentration required for a pressor response to rHuEPO in vitro. The infusion of rHuEPO was stopped after measurements were obtained for three consecutive 10-min experimental periods. During the three consecutive 10-min recovery periods, isotonic saline, instead of rHuEPO, was infused intravenously at a rate of 0.0656 ml/min, and urine samples were collected. Arterial blood samples were drawn 30 min after starting the rHuEPO infusion and at the end of the recovery period. An equivalent amount of fresh arterial blood drawn from a donor rabbit was transfused after collection of each 5-ml blood sample to prevent changes in renal blood flow. A similar procedure was followed in the control group, but the isotonic saline vehicle, instead of rHuEPO, was infused intrarenally at a rate of 0.0656 ml/ml for 30 min through the left renal artery catheter.

The glomerular filtration rate was estimated from the clearance of creatinine (Ccr). Plasma and urinary concentrations of creatinine were determined using an analyzer (VS-700S, Nihon Densi, Tokyo, Japan). Plasma and urinary concentrations of urea nitrogen, sodium, and potassium were determined using an autoanalyzer (STAT/ION-II, Nihon Technicon, Tokyo, Japan). Renal vascular resistance was calculated by dividing the mean arterial pressure by renal blood flow and expressed as mmHg per milliliter per minute. The filtered sodium load (FNa) and net tubular reabsorption of sodium (RNa) were calculated by multiplying the glomerular filtration rate by the plasma concentration of sodium and by subtracting the urinary sodium excretion from the filtered sodium load, respectively. These values are expressed as µeq/min.

Statistical Analysis
All data in figures and tables are expressed as mean ± SEM. Repeated measures analysis of variance (ANOVA) for overall differences and a two-sample t-test or Wilcoxon rank sum test was used to compare group differences. A p < 0.05 was considered to indicate statistical significance.

Results
Absolute values for the variables in the third basal period in the time control and rHuEPO groups are summarized in Table 1. During the third basal period, there were significant differences between the two groups in PNa, UV, UNa, and FENa, but not in mean arterial pressure (MAP), renal blood flow (RBF), renal vascular resistance (RVR), heart rate (HR), arterial hematocrit (Hct), renal plasma flow (RPF), glomerular filtration rate (GFR), filtration fraction (FF), UKV, FNa, RNa, or UNa-V.

Intrarenal infusion of rHuEPO at a rate of 100 U/min produced no significant changes in MAP, RBF or RVR (Fig. 1). There were no significant differences in these variables between the two groups in the recovery period. Heart rate did not change significantly in the control group, and no significant changes in the recovery period. There were no significant differences in these variables between the two groups in the recovery period. The plasma concentration of sodium was higher in the rHuEPO group than in the time control group in the third basal period but did not differ be-
between the two groups in the experimental or recovery periods. The plasma potassium concentration was not significantly different between the two groups during the experiment (data not shown). Although there was no significant difference between the two groups in MAP or renal hemodynamics in the basal period, significant differences were observed between the two groups in UV, UNaV, and FENa in the third basal period. Intrarenal infusion of rHuEPO produced a gradual and significant decrease in UV from the basal values at 10, 20 and 30 min, as compared with the time control group (Fig. 3). Even in the recovery period, a significant decrease in UV was observed.

Intrarenal infusion of rHuEPO produced significant decreases in UNaV from the basal values at 10, 20, and 30 min, as compared with the time control group (Fig. 3). Changes in UNaV during the recovery period were significant.

Intrarenal infusion of rHuEPO produced significant decreases in UKV from the basal values at 10, 20, and 30 min, as compared with the time control group. The decrease in UKV was still significant in the rHuEPO-treated group during the first recovery period. Intrarenal infusion of rHuEPO caused a significant decrease in FENa at 30 min, as compared

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### Table 1. Absolute Values for Various Parameters in the Third Basal Period

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>RBF (ml/min)</th>
<th>RVR (mmHg·ml/min)</th>
<th>HR (beat·min⁻¹)</th>
<th>Hct (%)</th>
<th>RPF (ml/min)</th>
<th>GFR (ml/min)</th>
<th>FF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time control (n=6)</td>
<td>118±3.1</td>
<td>43.8±4.4</td>
<td>2.85±0.32</td>
<td>268±8.2</td>
<td>40.8±1.4</td>
<td>26.0±3.0</td>
<td>5.05±0.59</td>
<td>19.9±2.2</td>
</tr>
<tr>
<td>rHuEPO (n=6)</td>
<td>118±4.0</td>
<td>44.7±3.0</td>
<td>2.70±0.20</td>
<td>274±14</td>
<td>40.2±1.4</td>
<td>26.8±2.1</td>
<td>5.36±0.34</td>
<td>20.5±1.8</td>
</tr>
</tbody>
</table>

Values are mean±SE; MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; HR, heart rate; Hct, arterial hematocrit; RPF, renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; PNa, plasma sodium concentration; PK, plasma potassium concentration; UV, urine volume; UNaV, urinary sodium excretion; UKV, urinary potassium excretion; FNa, filtered sodium load; PNa, net tubular reabsorption of sodium; FENa, fractional sodium excretion; UNaV, urinary excretion of urea nitrogen. *p<0.05 vs. the time control group.
with the time control group. Even in the recovery period, a significant decrease in FENa was observed. No significant difference was observed between the time control and rHuEPO groups in FNa, RNa, or UUNV in the basal, experimental, or recovery periods (Fig. 4). In the experimental and recovery periods, rHuEPO tended to increase RNa, but the increase was not significant.

**Discussion**

We previously demonstrated that this in vivo model was useful for study of the effects of vasoactive substances on renal circulation and function (9-11). Using this model, the present study was designed to determine whether rHuEPO has direct effects on renal hemodynamics or renal function in anesthetized rabbits without renal failure. The present results demonstrated that rHuEPO had no direct effects on MAP or renal hemodynamics in anesthetized rabbits without renal failure, but that it stimulated net tubular sodium reabsorption, possibly by a direct tubular effect, and reduced urine volume and urinary excretion of sodium and potassium.

Recombinant HuEPO has been found to reverse anemia in hemodialysis patients, but it has been associated with the development or worsening of hypertension in a considerable number of these patients (3, 5, 6, 12-14). The hypertension induced by rHuEPO has been attributed to an increase in blood viscosity, strongly related to the increase in hematocrit (4, 6), or to the vasoconstriction caused by improvement of hypoxia (3, 7). Heidenreich et al. (8) recently reported that rHuEPO had a direct vasoressor effect on renal and mesenteric resistance vessels of normotensive Wistar-Kyoto rats in vitro and that the threshold concentration required to increase the pressor response to rHuEPO was 10 U/ml. They suggested that the vasoressor activity of rHuEPO may contribute to the development of hypertension in rHuEPO-treated dialysis patients and may amplify or aggravate other mechanisms responsible for the development of hypertension.

In the present study, there were significant differences between the time control and rHuEPO groups in FNa, UV, UNaV, and FENa in the basal period. The reason for this is not clear. Body weight, surgical maneuver, priming volume of the 5% dextrose solution, and the time schedule for the experiment were not different between the two groups. Other conditions, such as sodium status and water intake, might have differed. There were no
significant differences between the time control and rHuEPO groups in MAP, RBF, RVR, GFR, FF, or Hct in the experimental period. The renal plasma concentration of rHuEPO, estimated by renal plasma flow, was higher than 27 U/ml, which exceeds the threshold concentration identified by Heidenreich et al. for a pressor response to rHuEPO in vitro. Radioimmunoassay measurements of plasma concentrations of human erythropoietin in normal individuals have ranged from 0.003 to 0.02 U/ml. When 3000 U of rHuEPO was administered three times weekly following dialysis, plasma concentrations of human erythropoietin ranged from 0.02 to 0.04 U/ml, which is much lower than the threshold concentration required for a pressor response to rHuEPO in vitro. Recombinant HuEPO has been found to increase the intracellular free calcium concentration in single early human erythroid precursors (15) and to induce differentiation and proliferation of erythroid cells. The threshold concentration of rHuEPO needed to induce an increase in intracellular free calcium was 0.2 U/ml, and the presence of extracellular calcium was not necessary to induce the observed increase in intracellular free calcium, suggesting that rHuEPO had no direct effects on MAP or renal hemodynamics, despite an rHuEPO-stimulated increase in intracellular free calcium concentration in renal vascular smooth muscle cells. Mechanisms other than a direct vasopressor effect, such as chronic stimulation of differentiation and proliferation of vascular smooth muscle cells, may be involved in the pathogenesis of hypertension induced by chronic rHuEPO administration.

Intrarenal infusion of rHuEPO produced decreases in UV, UrNaV, UrKv, and FENa, suggesting that it exerted a direct tubular effect that stimulated sodium reabsorption. However, possible effects of rHuEPO on humoral factors, such as angiotensin II and aldosterone, cannot be excluded. Increased urinary excretion of urea nitrogen (UrUN) has been found to be an indication of inhibition of tubular sodium reabsorption, related to washout in the medullary nephron (16). The lack of effect of the intrarenal rHuEPO infusion on UrUN in the present experiment further suggests the absence of rHuEPO-associated hemodynamic effects. The site of action of rHuEPO in the renal tubules remains to be defined.

In conclusion, the present study showed that rHuEPO had no direct effects on MAP or renal hemodynamics, but that it stimulated the tubular reabsorption of sodium, and reduced urine volume and urinary excretion of sodium and potassium in anesthetized rabbits without renal failure.

Acknowledgements
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References