Effects of Erythropoietin Administration on Blood Pressure and Urinary Albumin Excretion in Rats

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The effects of recombinant human erythropoietin (rHuEPO) administration on blood pressure and urinary albumin excretion were studied in normotensive Wistar-Kyoto rats (WKY), in spontaneously hypertensive rats (SHR), and in SHR rats treated with an angiotensin converting enzyme inhibitor (SHR-ACEi). Rats were housed in metabolic cages and treated with rHuEPO (150 U/kg body weight [bw] three times a week) for 6 weeks. Control animals received the vehicle only (0.25 mL of physiological saline). An angiotensin converting enzyme inhibitor was administered in the drinking water for 6 weeks (spirapril 5 mg/kg bw). Systolic blood pressure (SBP), and 24 h urinary albumin excretion (UAE) were measured once a week. No significant differences in SBP were observed between rHuEPO and vehicle-treated normotensive animals at the end of the treatment (171.9 ± 4.9 v 172.1 ± 5.6 mm Hg, respectively). After 6 weeks, SBP was significantly higher in SHR and SHR-ACEi groups treated with rHuEPO than in control groups (239.8 ± 7.3 and 243.0 ± 7.3 mm Hg v 218.1 ± 6.0 and 187.9 ± 4.6 mm Hg, respectively); UAE was significantly higher in groups treated with rHuEPO than in control groups (WKY: 265.9 ± 19.5 v 127.0 ± 12.3 μg/100 g bw, SHR: 1668.4 ± 564.6 v 234.8 ± 22.9 μg/100 g bw, and SHR-ACEi: 1522.7 ± 448.3 v 143.0 ± 18.9 μg/100 g bw, respectively). We concluded that erythropoietin treatment causes an increase in arterial pressure in SHR only, and an increase in UAE in both normotensive and hypertensive rats. The albuminuric effect was not entirely dependent on increased blood pressure. The treatment with an angiotensin converting enzyme inhibitor did not modify either the proteinuric or the pressor effects.   Am J Hypertens 1997;10:772-778 © 1997 American Journal of Hypertension, Ltd.

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It has been shown that the replacement therapy with recombinant human erythropoietin is effective in correcting the anemia of patients with chronic renal failure[1,2] and thus in abrogating blood transfusion requirements.[3–5] However, in a considerable proportion of erythropoietin-treated patients, development or worsening of arterial hypertension has been described.[6–8] These untoward effects have been ascribed to an increase of peripheral vascular resistances (due to the increased blood viscosity and to the suppression of the hypoxic vasodilatation), which is not appropriately counterbalanced by a nor-

mal readaptation of the cardiac output.[6,9–11] In patients with chronic renal failure, a positive history of familial hypertension or a preexisting hypertensive state may increase the incidence of hypertensive response to erythropoietin therapy.[12,13] Thus, it is likely that the hypertensive effect of erythropoietin treatment may be related either to an altered arterial pressure control associated to the chronic renal failure or to a genetic predisposition in developing arterial hypertension.

Although it is clear that in patients with chronic renal failure the erythropoietin treatment increases cardiovascular morbidity, possible adverse effects of erythropoietin therapy in other forms of anemia (not associated to impaired renal function) have never been investigated in spite of the increasing use of erythropoietin therapy in clinical practice.[14–17] It is also unknown whether a normal renal function may prevent the increase of blood pressure related to erythropoietin administration. In this regard, it is interesting to note that, when healthy subjects were treated with erythropoietin, blood pressure did not increase in spite of similar increases in hematocrit and hemoglobin.[18] On the other hand, a glomerular damage related to increases in hematocrit has been described in rats,[19,20] thus it would also be important to investigate whether erythropoietin treatment is associated with a specific renal damage.

To investigate whether the presence of intact kidneys can prevent the development or the worsening of arterial hypertension during erythropoietin treatment, the effect of erythropoietin administration on blood pressure was studied in both normotensive and spontaneously hypertensive rats. In addition, in the same animals, a possible untoward effect of erythropoietin treatment on the kidney was investigated by measuring 24-h urinary albumin excretion that is known to be an early index of glomerular damage.[21–23] Finally, in another group of hypertensive rats, we studied whether the hypotensive treatment with a converting enzyme inhibitor could prevent or modify the pressor and proteinuric effects of the erythropoietin treatment.

METHODS

The experiments were performed in conscious normotensive (Wistar-Kyoto, WKY) and spontaneously hypertensive (SHR) rats weighing 250 g. Animals were individually housed in metabolic cages in a temperature-controlled room at 22° to 24°C with a 12:12 light-dark cycle for 8 weeks. During the first 2 weeks, rats were accustomed to the metabolic cages and to the procedures of measuring systolic blood pressure (tail cuff method). After this period, basal values were collected for all variables then rats were treated with 150 U/kg body weight (bw) of human recombinant erythropoietin (rHuEPO), subcutaneously, three times a week for 6 weeks. Control animals received the

vehicle of rHuEPO (0.25 mL of physiological saline). In addition to vehicle or erythropoietin treatment, a group of spontaneously hypertensive rats was also treated with an angiotensin converting enzyme inhibitor (ACEi; spirapril) for 6 weeks (SHR-ACEi). Spirapril was diluted in the drinking water in a concentration appropriate to approximately obtain an intake of 5 mg/kg bw/day. According to treatment the six experimental groups were: WKY-EPO (n = 8), treated with rHuEPO; WKY-VEH (n = 8), treated with vehicle; SHR-EPO (n = 8), treated with rHuEPO; SHR-VEH (n = 8), treated with vehicle; SHR-ACEi-EPO (n = 8), treated with an ACEi and rHuEPO; and SHR-ACEi-VEH (n = 8), treated with ACEi and vehicle.

Once a week, systolic blood pressure, body weight, 24-h water intake and 24-h urine volume were measured by the same investigator who was unaware of the specific rat treatment. Systolic blood pressure was assessed by indirect pressure measurements (tail-cuff method) averaging six recordings performed in each animal. In our experimental conditions, it was verified that the tail cuff method overestimates values measured by direct intraarterial recordings by about 10%.[24] However, original, uncorrected values are reported in the results. Urinary sodium concentration was determined by potentiometric method. Urinary albumin concentration was measured by a radio immunoassay method, modified with the specific antibody for the albumin of rat. The 24-h urinary sodium and albumin excretion were calculated by multiplying urine volume times urine sodium and albumin concentrations, and normalized for body weight.

At the end of the sixth week of treatment animals were killed by decapitation and 5 mL of trunk blood were collected to measure hematocrit and hemoglobin concentration (colorimetric method). In addition, one kidney was removed, fixed in 10% buffered formalin, and paraffin embedded. Coronal sections (4 μm) were then cut and stained with hematoxylin/eosin and periodic acid-Schiff reaction for standard microscopic examination.

Statistical Analysis   Data are presented as means ± standard error of the mean (SEM). The time course of systolic blood pressure and urinary albumin excretion were analyzed by one-way analysis of variance (ANOVA) for repeated measures followed by the Scheffé F procedure to detect which treatment value was different from the basal one. Differences among control and erythropoietin treated groups for systolic blood pressure, urinary albumin excretion as well as for body weight, hematocrit, hemoglobin, water intake, urine volume, and urinary sodium excretion were analyzed by the unpaired t test. Differences among the values measured in WKY, SHR, and SHR-

TABLE 1. BASAL VALUES OF WATER INTAKE, URINE VOLUME, SODIUM EXCRETION, AND BODY
WEIGHT IN NORMOTENSIVE RATS (WKY), HYPERTENSIVE RATS (SHR), AND HYPERTENSIVE RATS
RECEIVING AN ANGIOTENSIN CONVERTING ENZYME INHIBITOR (SHR-ACEI)

	WKY		SHR		SHR-ACEi	
	VEH	EPO	VEH	EPO	VEH	EPO
Water intake (mL/24 h/100 g)	15.6 ± 0.6	14.6 ± 0.2	12.5 ± 0.5	11.7 ± 0.8	13.4 ± 0.6	12.7 ± 0.4
Urine volume (mL/24 h/100 g)	7.0 ± 0.8	5.5 ± 0.4	3.9 ± 0.5	3.9 ± 0.4	3.7 ± 0.3	4.0 ± 0.2
Sodium excretion (μEq/24 h/100 g)	662.4 ± 42.2	636.1 ± 28.1	418.5 ± 20.6	437.6 ± 28.9	497.7 ± 21.4	554.3 ± 24.6
Body weight (g)	242.8 ± 1.4	243.8 ± 3.2	272.1 ± 3.8	275.8 ± 5.5	243.8 ± 4.7	244.2 ± 4.9

Each value represents the mean ± SEM of eight animals. VEH, vehicle; EPO, erythropoietin.

ACEi groups, both in the vehicle or erythropoietin treatment groups, were analyzed by factorial analysis of variance followed by the Scheffé F procedure for post hoc comparison. After logarithmic transformation of the urinary albumin excretion values, simple regression analysis was performed between urinary albumin excretion and arterial pressure and hematocrit values.

RESULTS

Effects of Erythropoietin Treatment on Body Weight, Hematocrit, Hemoglobin, Water Intake, Urine Volume, and Urinary Sodium Excretion Before any pharmacological treatment, no differences in body weight, water intake, urine volume, and urinary sodium excretion were observed between the control and rHuEPO treated groups. In hypertensive animals, under basal conditions, water intake, urine volume, and urinary sodium excretion were significantly lower than values observed in normotensive animals (see Table 1). As shown in Figures 1 and 2, after 6 weeks of treatment with erythropoietin, there were no differences in body weight, water intake, urine volume, and urinary sodium excretion between control and erythropoietin treated animals. Hematocrit and hemoglobin concentrations were significantly higher in the erythropoietin treated animals than in control rats (Figure 1). These hematologic effects were equal in normotensive and hypertensive rats, and they were not modified by the concomitant administration of the angiotensin converting enzyme inhibitor. Treatment with ACEi increased urinary sodium excretion in SHR rats to values similar to those of WKY rats independently from the administration of erythropoietin (Figure 2).

Effect of Erythropoietin Treatment on Blood Pressure and Urinary Albumin Excretion in WKY Rats Although data were collected every week, values shown in Table 2 refer to four measurements being representative of the whole time course. In basal conditions before any pharmacological treatment, systolic blood pressure of the WKY-EPO and WKY-VEH groups was similar. Subsequently, arterial pressure increased in both groups of animals slightly but not significantly. At no time were significant differences in systolic arterial pressure between control and erythropoietin groups observed.

Under basal conditions, urinary albumin excretion was higher in the WKY-EPO group than in the WKY-VEH group, but this difference was not statistically significant. After the second week erythropoietin treatment caused a progressive increase in urinary albumin excretion so that, after 6 weeks of treatment, urinary albumin excretion values were significantly larger than basal values. On the contrary, in the control group, urinary albumin excretion was unchanged or slightly decreased during the 6 weeks of treatment. Furthermore, after the second week of treatment, urinary albumin excretion of the erythropoietin-treated group was significantly larger than urinary albumin excretion of the control group.

Effect of Erythropoietin Treatment on Blood Pressure and Urinary Albumin Excretion in SHR Rats In basal conditions (Table 2), there were no differences in systolic blood pressure and urinary albumin excretion between the SHR-VEH and SHR-EPO groups. During the following weeks, systolic arterial pressure slightly, but not significantly, increased in the SHR-VEH group. In the SHR-EPO group systolic blood pressure markedly increased, and it became significantly higher than blood pressure of the control group after the second week of treatment with erythropoietin.

In the SHR-VEH group, during the 6 weeks of the experimental period, urinary albumin excretion slightly increased and it was significantly larger than basal values at the sixth week. In the erythropoietin-treated rats, urinary albumin excretion markedly in-
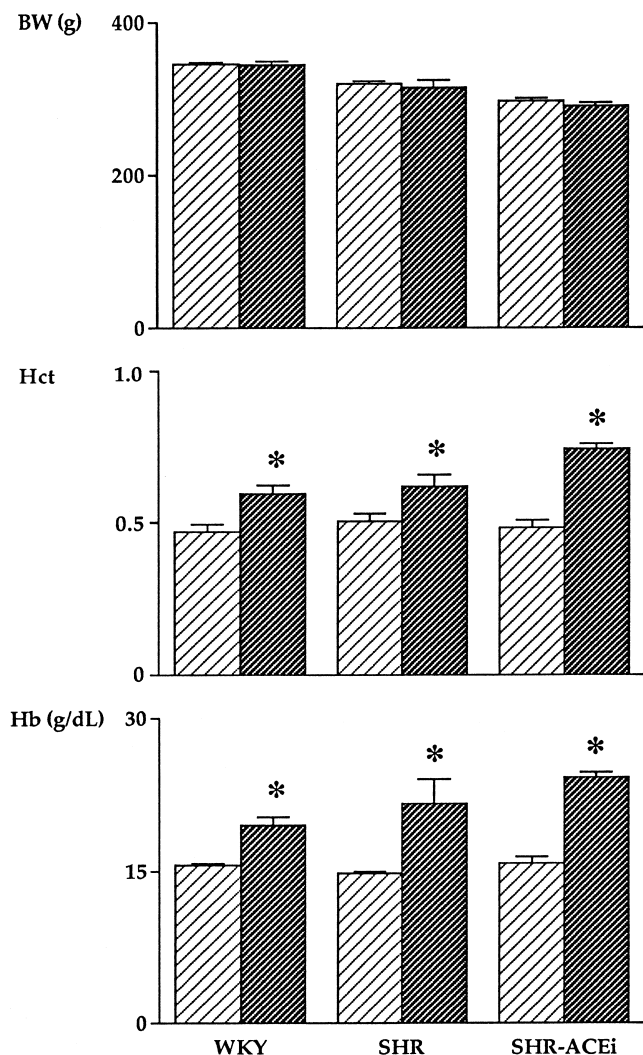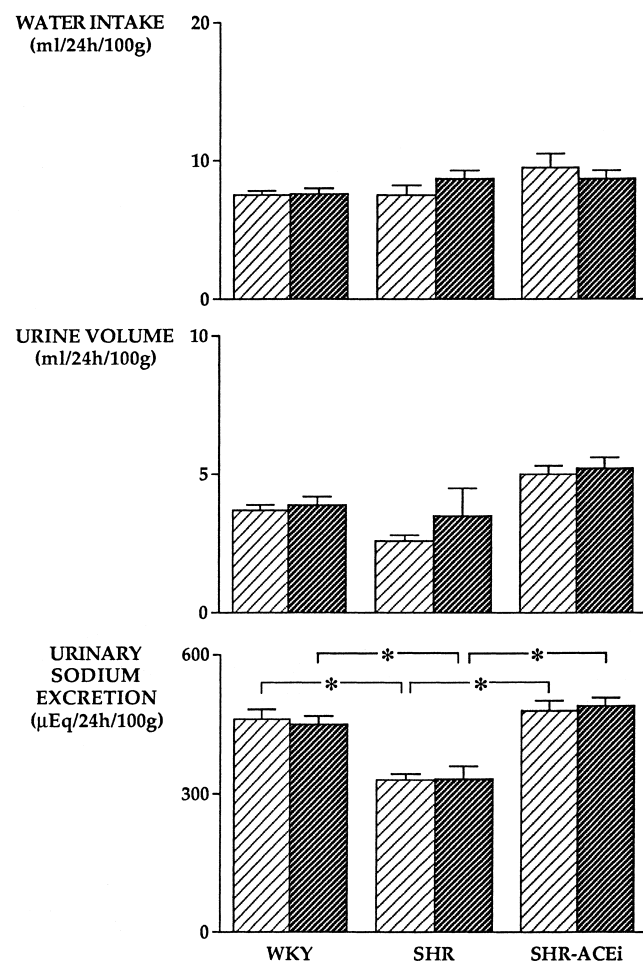
FIGURE 1. *Graphs showing the values of body weight (BW), hematocrit (Hct), and hemoglobin (Hb) measured after 6 weeks of treatment with erythropoietin (heavy shading) or vehicle (light shading) in normotensive (WKY), hypertensive (SHR), and angiotensin converting enzyme inhibitor treated hypertensive rats (SHR-ACEi). Each bar represents the mean ± SEM of eight animals. *P < .05 between erythropoietin and vehicle treated animals (unpaired t test).*

converting enzyme inhibitor, systolic blood pressure of the SHR-ACEi-VEH group significantly decreased and it remained lower than basal values for the whole period of treatment. In animals simultaneously treated with ACEi and erythropoietin, systolic blood pressure progressively increased and, after the second week of treatment, it was significantly higher than in the control group.

Under basal conditions, urinary albumin excretion was higher in the SHR-ACEi-EPO group than in the SHR-ACEi-VEH group, but this difference did not reach the statistical significance. During the following weeks, in rats treated with ACE only, a slight, but not significant, decrease of urinary albumin excretion was observed. In the SHR-ACEi-EPO group, the erythropoietin treatment caused a large increase of urinary

FIGURE 2. *Graphs showing the values of water intake, urine volume, and urinary sodium excretion measured after 6 weeks of treatment with erythropoietin (heavy shading) or vehicle (light shading) in normotensive (WKY), hypertensive (SHR), and angiotensin converting enzyme inhibitor hypertensive rats (SHR-ACEi). Each bar represents the mean ± SEM of eight animals. *P < .05 v WKY and SHR-ACEi animals (factorial ANOVA + Scheffé F procedure).*

creased and it was significantly larger than in SHR-VEH rats after the second week of treatment. At the end of the pharmacological treatment, urinary albumin excretion was eightfold higher in the SHR-EPO group than in the SHR-VEH rats.

Effect of Erythropoietin on Blood Pressure and Urinary Albumin Excretion in SHR-ACEi Groups   In basal conditions (Table 2), before any pharmacological treatment, systolic arterial pressure of the SHR-ACEi-VEH group was slightly, but not significantly, higher than the arterial pressure of the SHR-ACEi-EPO group. During the second week of treatment with the

TABLE 2. EFFECTS OF ERYTHROPOIETIN TREATMENT ON SYSTOLIC BLOOD PRESSURE AND URINARY ALBUMIN EXCRETION IN NORMOTENSIVE (WKY), HYPERTENSIVE RATS (SHR), AND HYPERTENSIVE RATS RECEIVING AN ANGIOTENSIN CONVERTING ENZYME INHIBITOR (SHR-ACEI)

	WKY		SHR		SHR-ACEi	
	VEH	EPO	VEH	EPO	VEH	EPO
SBP (mmHg)						
Basal	162.4 ± 5.8	159.4 ± 6.1	209.0 ± 4.2	204.4 ± 4.2	212.4 ± 2.1	204.3 ± 4.6
Week 2	167.8 ± 6.7	158.8 ± 7.7	204.6 ± 3.2	219.3 ± 3.8†	192.4 ± 5.4*	203.4 ± 4.0
Week 4	169.4 ± 7.0	172.6 ± 6.5	215.5 ± 5.7	242.1 ± 6.0*†	205.6 ± 3.9	230.4 ± 5.1*†
Week 6	172.1 ± 5.6	171.9 ± 4.9	218.1 ± 8.2	239.8 ± 7.3*†	187.9 ± 4.6*	243.0 ± 7.3*†
UAE (μg/24 h/ 100 g)						
Basal	138.2 ± 12.5	175.2 ± 15.5	145.6 ± 14.8	156.4 ± 11.4	176.2 ± 14.4	253.9 ± 56.3
Week 2	100.8 ± 7.7	128.1 ± 8.0†	151.9 ± 9.6	195.5 ± 7.8†	170.7 ± 11.8	285.7 ± 64.0
Week 4	90.8 ± 6.5*	186.9 ± 13.7†	200.6 ± 22.7	870.3 ± 248.7†	133.7 ± 21.0	730.3 ± 173.4†
Week 6	127.2 ± 12.3	265.9 ± 19.5*†	234.8 ± 22.9*	1668.4 ± 564.6*†	143.0 ± 18.9	1522.7 ± 448.3*†

Each value represents the mean ± SEM of eight animals. SBP, systolic blood pressure; UAE, urinary albumin excretion normalized to body weight; VEH, vehicle; EPO, erythropoietin.

* P < .05 versus the corresponding basal value; †P < .05 between erythropoietin and vehicle treated animals.

albumin excretion that was significantly higher than control values after the fourth week of treatment. At the end of the pharmacological treatment, urinary albumin excretion was tenfold higher in the erythropoietin treated than in the control rats.

Histologic Findings At least two coronal sections of each kidney were examined nonquantitatively by light microscopy. The examiner was not aware of the strain or treatment of the rat. No differences were detected in the number of lesions between control and erythropoietin treated rats in either the SHR or WKY groups.

Correlations Between Arterial Pressure and Urinary Albumin Excretion In spontaneously hypertensive rats, treated or not with ACEi, the administration of erythropoietin caused an increase of both the systolic arterial pressure and the urinary albumin excretion. In these animals a significant correlation between systolic arterial pressure and urinary albumin excretion was found (Figure 3).

DISCUSSION

These experiments show that the erythropoietin treatment elicits an increase in blood pressure in spontaneously hypertensive rats only. Despite similar increases in hematocrit and, therefore, in blood viscosity, arterial pressure does not change in normotensive rats that underwent erythropoietin treatment. Our data also demonstrate that the erythropoietin administration causes a significant increase in urinary albumin excretion not only in spontaneously hypertensive animals but also in normotensive rats.

Our results on the hypertensive effect of erythropoietin are in agreement with pervious data by Susic and associates[25] showing that upward and downward chronic hematocrit changes did increase or decrease blood pressure in hypertensive rats only, whereas the same maneuvers did not affect blood pressure in normotensive rats.

An increase in hematocrit may influence hemodynamics either by a direct effect on blood viscosity, and consequently on total peripheral resistances, or by an indirect effect on local vascular tone, due to an increase of oxygen delivery to tissues resulting from the increased hemoglobin blood concentration. Indeed, an increase in hemoglobin concentration may decrease nitric oxide production from endothelial cells.[26,27] Nevertheless, the pressor response to erythropoietin treatment, being evident in SHR strain only, appears to be dependent on conditions of altered blood pressure control. The precise mechanism underlying this phenomenon remains unclear. An altered control of arterial pressure may be genetically determined or the consequence of the cardiovascular damage due to the preexisting hypertensive state. It is also possible that spontaneously hypertensive rats are not able to appropriately decrease cardiac output when peripheral vascular resistances increase. As described in patients who develop pressor response to erythropoietin therapy,[28] spontaneously hypertensive rats might have an increased vascular reactivity to changes in oxygen delivery. Besides an increase in hematocrit, other mechanisms may be implicated to explain the increase in peripheral vascular resistances following erythropoietin treatment. In an in vitro model it has been suggested that erythropoietin exerts a direct vasopressor effect on isolated proximal resistance vessels of the kidney and mesenteric vasculature[29]; it was shown
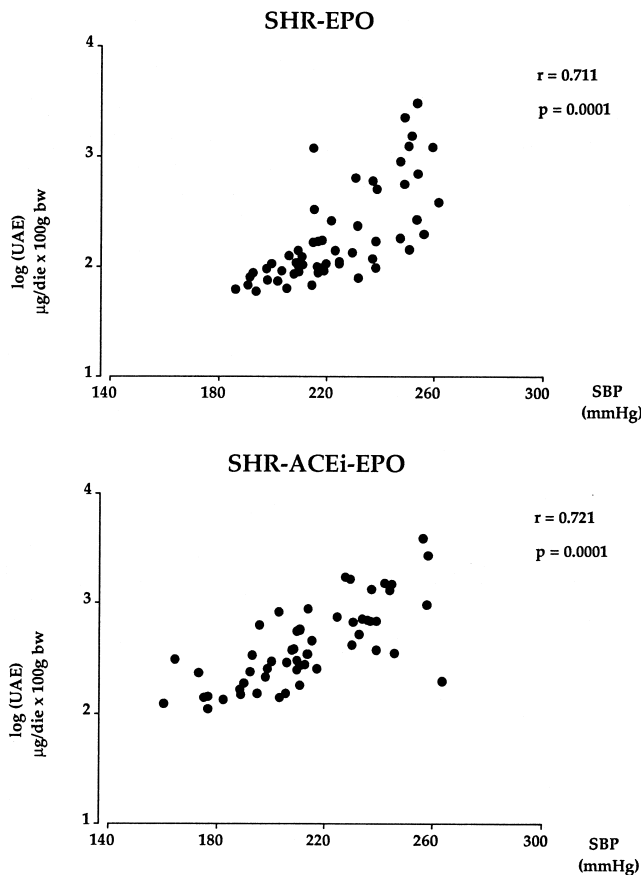
FIGURE 3. *Scattergrams showing the relation between the values of systolic blood pressure (SBP) and urinary albumin excretion (UAE) measured during 6 weeks of treatment with erythropoietin in hypertensive (SHR n = 56) and angiotensin converting enzyme inhibitor treated hypertensive rats (SHR-ACEi n = 56). The correlation coefficient (r) and the significance (P) are shown for each scattergram.*

also that erythropoietin treatment induces stimulation of endothelin 1.[30] Recently, Hand and colleagues[31] have demonstrated that erythropoietin treatment increases the impaired vascular responsiveness to norepinephrine in hemodialysis patients. Although it has been suggested that the renin-angiotensin system may contribute to the pathogenesis of the hypertensive effect of erythropoietin administration,[32,33] our results do not confirm the possibility that the renin angiotensin system may play an important role in mediating the pressor effect of the erythropoietin administration. Indeed treatment with an ACEi does not prevent the increase of arterial pressure following erythropoietin treatment. In our experimental conditions, a role of the renin-angiotensin system seems limited to mediate the sodium retention in hypertensive rats, as ACEi treatment significantly increased urinary sodium excretion to a level equal to that observed in normotensive rats. In addition our data indicate that, a normal renal function does not

prevent the increase of blood pressure related to erythropoietin administration.

The increase in urinary albumin excretion, following the erythropoietin treatment, can be the consequence of the concomitant increase in blood pressure, as suggested by the positive correlation between arterial pressure and urinary albumin excretion observed in hypertensive rats. On the other hand, in normotensive rats treated with erythropoietin no correlation between arterial pressure and urinary albumin excretion was found, because urinary albumin excretion increased in spite of no changes in arterial pressure. Mechanisms other than arterial pressure should be claimed to explain the increased urinary albumin excretion observed in normotensive animals. As it has been demonstrated that both acute and chronic changes in hematocrit can impair renal and glomerular function,[20] it is likely that the erythropoietin-induced increase in hematocrit may play a role in mediating the increased urinary albumin excretion. Indeed, a significant correlation between hematocrit and urinary albumin excretion was found at the sixth week of treatment, when all erythropoietin-treated animals (both normotensive and hypertensive rats) were analyzed (data not shown). Although our experiments do not establish the mechanisms by which an increase in hematocrit promotes albuminuria, a reasonable explanation might be an increase in glomerular pressure secondary to the increased hematocrit.[20] Thus, it is possible that both arterial pressure and hematocrit increases elicit the albuminuric effect by the same mechanism: an increase in glomerular pressure. ACEi treatment at a dosage that was effective in preventing the spontaneous increase in arterial pressure and urinary albumin excretion did not modify the proteinuric effect caused by the erythropoietin administration. This finding suggests that angiotensin II is not the major mechanism mediating the glomerular effect of erythropoietin treatment.

Microalbuminuria is considered to be an index of glomerular vascular damage.[21,22] Our data, by indicating that erythropoietin treatment increased urinary albumin excretion also in normotensive animals with a normal renal function, suggest that urinary albumin excretion might be a sensitive and early marker of vascular damage during erythropoietin treatment.

In conclusion, our results show that the hypertensive effect of erythropoietin treatment, likely mediated by the increased viscosity, is evident in hypertensive rats only. The administration of erythropoietin causes an increase of urinary albumin excretion that is not entirely dependent on the concomitant increase in arterial pressure, both in hypertensive and normotensive rats. Other mechanisms, such as the increased hematocrit, might explain this side effect.

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