Role of erythropoietin and nitric oxide in modulating the tone of human renal interlobular and subcutaneous arteries from uraemic subjects

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ABSTRACT

This study investigated potential reasons why erythropoietin (EPO) given therapeutically to patients with renal failure may increase peripheral, but not renal, vascular resistance. This was done by comparing the effects of EPO on resting tension in normal renal interlobular and subcutaneous vessels from uraemic patients. In human subcutaneous arteries from uraemic subjects, noradrenaline- and KCl-induced vasoconstrictions were enhanced when nitric oxide (NO) production was blocked with \( N^G \)-nitro-L-arginine methyl ester (L-NAME), but were unaffected by EPO, while acetylcholine- and bradykinin-induced concentration-dependent relaxations were markedly attenuated by L-NAME, but not by EPO. The noradrenaline- and KCl-induced vasoconstrictions of human renal interlobular arteries were unaffected by the presence of L-NAME, but were attenuated by EPO (20 units \( \text{mL}^{-1} \)) by some 33\% \( (P < 0.01) \); this effect was enhanced by the co-administration of L-NAME. Acetylcholine and bradykinin caused comparable dilatations of the interlobular arteries; the response to the former was attenuated by L-NAME, but none of these responses were changed by EPO. EPO given alone, at a concentration of either 0.1 or 20 units \( \text{mL}^{-1} \), had no effect on basal resting tone. NO production mediated both acetylcholine- and bradykinin-induced relaxation in this vessel type. In contrast, in the interlobular arteries there was no indication of NO modulating the level of vasoconstriction, and it only mediated acetylcholine-induced dilation. These acute responses to EPO only partially explain its differential effects on the vasculature in renal failure.

INTRODUCTION

There has been long-standing concern over the use of erythropoietin (EPO) therapeutically, in that administration of the hormone to patients with chronic renal failure and to individuals requiring haemodialysis causes hypotension and may exacerbate pre-existing hypertension. The reasons for this are unclear, but a number of possibilities have been proposed; these include a rapid increase in haematocrit [1], a low baseline haematocrit

Key words: erythropoietin, human renal interlobular arteries, KCl, nitric oxide, \( N^G \)-nitro-L-arginine methyl ester (L-NAME), noradrenaline, uraemic subcutaneous arteries.

Abbreviations: EDRF, endothelium-dependent relaxation factor; EPO, erythropoietin; ET-1, endothelin-1; L-NAME, \( N^G \)-nitro-L-arginine methyl ester; NOS, nitric oxide synthase; \( pEC_{50} \) = \( -\log EC_{50} \); PSS, physiological saline solution; KPSS, PSS with equimolar substitution of KCl for NaCl, resulting in final potassium concentration of 125 mM.

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prior to EPO administration [2], the high doses as well as the intravenous route of administration of the hormone [1], the presence of native kidneys [3,4], a genetic predisposition to hypertension [5] and possibly the young age of the patients [6]. There are reports showing that the increase in blood pressure is independent of the increase in haematocrit in some patients [7]. What is puzzling in the therapeutic use of EPO in renal failure patients is that, although there is an increase in total peripheral resistance, indicative of increased vascular tone in most vascular beds, the residual renal haemodynamic function, in terms of stabilizing the gradual rise in plasma creatinine and a decrease in endogenous creatinine clearance rate, is preserved, suggesting that the renal vasculature is insensitive to this action of the hormone. Importantly, the use of EPO in other disease states in which anaemia is present has no effect on blood pressure.

Investigations are now being focused on a possible action of EPO at the tissue and cellular level, to study whether it may alter vascular characteristics. It has been reported that local injection of EPO caused a reduction in skeletal muscle blood flow [8], consistent with a direct action on the vasculature, and indeed EPO receptors have been shown to be present. However, the only in vitro study using normal human subcutaneous arteries failed to demonstrate a direct action of EPO on the resting tone of the vessels. This may not be surprising, as EPO therapy in patients with anaemia from other causes [9] does not cause hypertension. The situation becomes more complex in chronic renal failure, in that there is an accumulation of factors in the plasma that may also modify and potentiate the vascular responsiveness to various vasoactive agonists. To date, there has been little, if any, study of subcutaneous arteries taken from uraemic individuals. A further consideration is that EPO may have an action at endothelial cells, whereby it may modify the production of paracrine and autocrine factors, such as nitric oxide (NO) and endothelin, which indirectly could alter basal vascular tone as well as responsiveness to vasoactive compounds.

There is currently very little information in the literature about the pharmacological properties of human renal interlobular arteries in vitro, or indeed on their responses to EPO. These vessels are of particular interest, as they are located anatomically in an area close to the site of EPO synthesis and may be responsive to high levels of the hormone. Therefore an important element was to establish the physiological and pharmacological characteristics of these vessels.

The objectives of the present study were two fold: first, to determine how the contractile characteristics of resistance vessels taken from uraemic patients, which are known to contribute to EPO-induced hypertension, are modified as a consequence of acute exposure to EPO; and secondly, to assess the pharmacological properties of normal human renal interlobular arteries and to determine whether EPO has direct or indirect actions on these vessels. Because kidneys from patients with renal failure appear to be insensitive to EPO and because very little tissue would be available, it was considered pertinent to examine renal vessels from normal individuals. In this way, it was hoped that a clearer understanding could be achieved of the way in which EPO might exert its effect at the level of the vasculature.

**METHODS**

**Preparation of vessels**

Subcutaneous arteries \( (n = 103; \text{internal diameter } 133.1 \pm 10.7 \mu m \text{ (mean \pm S.E.M.)}) \) were obtained from 27 uraemic patients \( (\text{age } 47 \pm 10 \text{ years}; \text{range } 25–73 \text{ years}; 18 \text{ males/nine females}) \) who had not previously received EPO, and who were undergoing routine surgery for insertion of a continuous ambulatory peritoneal dialysis catheter. Renal interlobular arteries \( (n = 101; \text{mean internal diameter } 154.5 \pm 12.3 \mu m) \) were taken from the normal parts of kidneys that had been removed because of localized renal tumours \( (20 \text{ patients; twelve males/eight females; age } 60.0 \pm 5.0 \text{ years; range } 4–87 \text{ years}) \). The study was approved by the institutional Ethical Committee.

Arteries were placed in ice-cold \( (4^\circ \text{C}) \) physiological saline solution \( (\text{PSS; } 119 \text{ mM NaCl, } 4.7 \text{ mM KCl, } 2.5 \text{ mM CaCl}_2, 1.17 \text{ mM MgSO}_4, 25 \text{ mM NaHCO}_3, 1.18 \text{ mM KH}_2\text{PO}_4, 0.02 \text{ mM EDTA, } 5.5 \text{ mM glucose, pH } 7.4 \text{ at } 37^\circ \text{C}) \). The arteries were identified and dissected free of surrounding connective tissue. The vessels were mounted on \( 40 \mu m \text{ tungsten wires in a small-vessel myograph (Cambustion, Cambridge, U.K.) capable of measuring isometric tension. The arteries were bathed in PSS at } 37^\circ \text{C, bubbled with } 5\% \text{ CO}_2 \text{ in } \text{O}_2. \text{ Maximal potassium-induced activation was achieved by incubating in KPSS (PSS with equimolar substitution of KCl for NaCl, resulting in final potassium concentration of } 125 \text{ mM).} \)

After an equilibration period of \( 1 \text{ h in PSS, the passive tension/internal circumference characteristics were determined [10]. The vessels were then set to an internal circumference equivalent to } 90\% \text{ of that which they would have had when relaxed in situ under a transmural pressure of } 100 \text{ mmHg. The maximum active tension is developed at approximately this circumference [10]. The vessels were then maximally contracted for } 2 \text{ min every } 10 \text{ min on four occasions. The first two contractions were induced by KPSS and then } 5 \mu M \text{ noradrenaline, followed by } 5 \mu M \text{ noradrenaline and finally KPSS. Any vessels that failed to develop a maximum tension equivalent to a pressure of } 100 \text{ mmHg were discarded.} \)
Experimental protocol
Cumulative concentration–response curves were constructed sequentially for noradrenaline (0.01–10 μM), KCl (12–125 mM), acetylcholine (0.01–100 μM) (arteries pre-contracted with 3 μM noradrenaline), bradykinin (0.001–3 μM) and sodium nitroprusside (0.1 μM–2 mM).
Each concentration of agonist was left in contact with the vessels for 2 min, with the tension being recorded at the end of the period. Following each concentration–response curve, the vessels were washed with PSS and rested in PSS for 10 min. Groups of vessels (between six and eleven in each group) were then incubated with 0.1 or 20 units ml⁻¹ EPO for 1 h, with 0.1 mM Nω-nitro-L-arginine methyl ester (L-NAME) for 30 min, or with a combination of 20 units ml⁻¹ EPO and 0.1 mM L-NAME (EPO given alone for 30 min then L-NAME added for the subsequent 30 min). After these treatments, concentration–response curves were repeated for the above agonists in the presence of the appropriate compounds or combination of agents. Each artery was exposed to no more than three kinds of agonists. For investigations with endothelin-1 (ET-1; 0.001–0.2 μM), other sets of vessels were used and cumulative concentration–response curves were generated in the absence or presence of L-NAME or EPO.
In order to exclude the possibility of changes in sensitivity of the vessels with repeated exposure, timed controls were performed. Concentration–response curves were obtained for noradrenaline, KCl, acetylcholine, bradykinin and sodium nitroprusside. The vessels were washed in PSS and allowed to rest for 10 min, and the concentration–response curves were then repeated.

Chemicals
Acetylcholine, bradykinin, ET-1, L-NAME and noradrenaline were purchased from Sigma (Poole, Dorset, U.K.). Sodium nitroprusside was from David Bull Laboratories. EPO was obtained from Janssen-Cilag. Other reagents were purchased from BDH (Poole, Dorset, U.K.). All reagents were of analytical grade, and solutions were prepared fresh daily.

Statistical analysis
The negative log of the EC₅₀ value (pEC₅₀) was calculated using a computerized curve-fitting software package (Graphpad Inplot version 3.14). Where curve fitting was not appropriate, the maximum responses were compared. Relaxations in response to acetylcholine, bradykinin and sodium nitroprusside were expressed as percentages of the pre-contraction to noradrenaline. Contractions in response to noradrenaline, KCl and ET-1 were expressed as active tension (mN·mm⁻¹). All values are given as means±S.E.M. Differences between means were assessed using Student’s t test for paired or unpaired data as appropriate. The concentration–response curves were compared using two-factor ANOVA (analysis of variance). P < 0.05 was taken as statistically significant.

RESULTS
Subcutaneous arteries from uraemic subjects
Administration of EPO, over the concentration range 10–300 units ml⁻¹, into the bath had no effect on the resting tension of the vessels, which had a maximum value of 0.01 ± 0.3 mN·mm⁻¹ (n = 6).
Noradrenaline induced concentration-dependent contractions of the vessels, with a maximum response at around 2 μM noradrenaline. Repetition of the concentration–response curve as a timed control led to a significant shift of the curve to the right (P < 0.001), with a small but inconsistent reduction in the maximum response and a decrease in the pEC₅₀ (P < 0.01; Table 1). In contrast, incubation of the vessels with L-NAME resulted in a significant shift of the curve to the left when compared with the time control (P < 0.001), and this occurred along with a small increase in the maximum contraction of 10% and decrease in the pEC₅₀ (Table 1). EPO, at both 0.1 and 20 units ml⁻¹, resulted in a shift to the right of the concentration–response curve (Table 1). However, in neither case was this change significantly greater than that seen with the time control.
KCl induced concentration-dependent contractions of the vessels, with a maximum response at around 0.125 M KCl. Repetition of the concentration–response curve as a time control led to a shift to the right of the curve (P < 0.001), with a reduction in both the maximum response (by 16%; P < 0.01) and the pEC₅₀ (by 7%; P < 0.05) (Table 1). In contrast, incubation with L-NAME significantly enhanced the contraction in response to KCl, leading to a shift to the left of the concentration–response curve relative to its paired and time controls (P < 0.05 and P < 0.001 respectively), with an increase in the maximum contraction (by 46%; P < 0.05) at a time when pEC₅₀ did not change (Table 1). EPO, at both 0.1 and 20 units ml⁻¹, led to a shift to the right of the concentration–response curve relative to its paired control, but this change was not different from that seen with the time control (Table 1).
ET-1 induced concentration-dependent contractions, with a maximum response of 4.32 ± 0.37 mN·mm⁻¹ at around 0.2 μM and a pEC₅₀ of 9.05 ± 0.09 M. This response was increased in the presence of L-NAME by 53% (6.69 ± 1.27 mN·mm⁻¹), with a decrease in pEC₅₀ (8.40 ± 0.22 M; P < 0.05). In the presence of the low concentration of EPO, the sensitivity of the vessels to ET-1 decreased (pEC₅₀ 8.31 ± 0.22 M; P < 0.05), along with a reduction in the maximum constriction in response to ET-1 of 29% (maximum tension 3.08 ± 0.61
mN·mm⁻¹; *P < 0.05). This effect was not seen following exposure to the high EPO concentration (maximum tension 4.27 ± 0.12 mN·mm⁻¹; pEC₅₀ 8.89 ± 0.13 M).

Vessels were pre-contracted with noradrenaline and then exposed to acetylcholine, which induced relaxation reaching a maximum at 3 μM acetylcholine. Repetition of the concentration–response curve led to a small, but statistically significant, shift to the right of the curve (P < 0.001), with a significant decrease in pEC₅₀ (P < 0.05; Table 2). Incubation with l-NAME was associated with a decrease in both maximum relaxation (by 26%; P < 0.001) and pEC₅₀, (by 11%; P < 0.05) (Table 2). EPO at both 0.1 and 20 units·ml⁻¹ had no effect on acetylcholine-induced relaxation relative to the time or paired controls (Table 2).

Bradykinin induced a concentration-dependent relaxation of the vessels, with a maximum response at around 30 μM bradykinin. Repetition of the concentration–response curve did not lead to a change in either the pattern or the magnitude of the relaxation obtained (Table 2). Incubation with l-NAME attenuated the bradykinin-induced relaxation, with a maximum relaxation of only 21% (P < 0.05; Table 2). EPO at both 0.1 and 20 units·ml⁻¹ had no effect on bradykinin-induced relaxation (Table 2).

Sodium nitroprusside induced concentration-dependent relaxation of the vessels, with a maximum response at around 0.1 mM. Repetition of the concentration–response curve, as a timed control, was not associated with a significant change in the relaxation observed (Table 2). Pre-incubation with EPO at 0.1 or 20 units·ml⁻¹ had no effect on sodium nitroprusside-induced relaxation (Table 2).

**Human renal interlobular arteries**

Vessels were incubated with increasing doses of EPO, with exposure for 2 min at each dose. EPO over the concentration range 10–300 units·ml⁻¹ did not induce any contraction of the resting vessels, which developed a maximum tension of 0.02 ± 0.1 mN·mm⁻¹ (n = 6).

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**Table 1** Maximum tension and pEC₅₀ values for contractile responses for human subcutaneous arteries from uraemic subjects

For each protocol, n = 6–11 vessels derived from 2–5 subjects. *P < 0.05; **P < 0.01; ***P < 0.001 compared with value before treatment.

<table>
<thead>
<tr>
<th></th>
<th>Noradrenaline</th>
<th>KCl</th>
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<tr>
<td></td>
<td>Max. tension</td>
<td>pEC₅₀ (M)</td>
</tr>
<tr>
<td>Control</td>
<td>2.49 ± 0.32</td>
<td>6.05 ± 0.22</td>
</tr>
<tr>
<td>Time control</td>
<td>1.98 ± 0.17</td>
<td>6.40 ± 0.08*</td>
</tr>
<tr>
<td>Before l-NAME</td>
<td>3.98 ± 0.59</td>
<td>6.06 ± 0.06</td>
</tr>
<tr>
<td>+ l-NAME</td>
<td>4.38 ± 0.83</td>
<td>5.76 ± 0.07***</td>
</tr>
<tr>
<td>Before EPO</td>
<td>3.67 ± 0.25</td>
<td>6.56 ± 0.21</td>
</tr>
<tr>
<td>+ EPO (0.1 unit·ml⁻¹)</td>
<td>2.56 ± 0.26***</td>
<td>6.67 ± 0.13</td>
</tr>
<tr>
<td>Before EPO</td>
<td>3.34 ± 0.40</td>
<td>6.07 ± 0.17</td>
</tr>
<tr>
<td>+ EPO (20 units·ml⁻¹)</td>
<td>2.29 ± 0.50</td>
<td>6.21 ± 0.23</td>
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**Table 2** Maximum responses and pEC₅₀ values for relaxation of human subcutaneous arteries from uraemic subjects

For each protocol, n = 6–11 vessels derived from 2–5 subjects. *P < 0.05; ***P < 0.001 compared with value before treatment.

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholine</th>
<th>Bradykinin</th>
<th>Sodium nitroprusside</th>
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<tr>
<td></td>
<td>Max. response (%)</td>
<td>pEC₅₀ (M)</td>
<td>Max. response (%)</td>
</tr>
<tr>
<td>Control</td>
<td>91.9 ± 2.27</td>
<td>6.84 ± 0.19</td>
<td>85.5 ± 4.77</td>
</tr>
<tr>
<td>Time control</td>
<td>88.2 ± 5.90</td>
<td>6.42 ± 0.11*</td>
<td>87.6 ± 7.63</td>
</tr>
<tr>
<td>Before l-NAME</td>
<td>89.8 ± 2.87</td>
<td>7.26 ± 0.11</td>
<td>73.5 ± 7.16</td>
</tr>
<tr>
<td>+ l-NAME</td>
<td>66.6 ± 5.48***</td>
<td>6.43 ± 0.18*</td>
<td>57.8 ± 9.35*</td>
</tr>
<tr>
<td>Before EPO</td>
<td>86.6 ± 5.05</td>
<td>6.86 ± 0.21</td>
<td>88.3 ± 1.19</td>
</tr>
<tr>
<td>+ EPO (0.1 unit·ml⁻¹)</td>
<td>79.7 ± 5.56</td>
<td>7.19 ± 0.17</td>
<td>83.3 ± 4.04</td>
</tr>
<tr>
<td>Before EPO</td>
<td>91.2 ± 3.58</td>
<td>7.22 ± 0.09</td>
<td>89.8 ± 1.76</td>
</tr>
<tr>
<td>+ EPO (20 units·ml⁻¹)</td>
<td>83.9 ± 2.93</td>
<td>7.17 ± 0.36</td>
<td>89.6 ± 1.78</td>
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</table>
Table 3  Maximum tensions and pEC_{50} values for contractile responses of human renal interlobular arteries
For each protocol, n = 6–11 vessels derived from 2–5 subjects. *P < 0.05; **P < 0.01; ***P < 0.001 compared with value before treatment.

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<tr>
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<th>Noradrenaline</th>
<th>KCl</th>
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<tr>
<td></td>
<td>Max. tension (mN · mm⁻¹)</td>
<td>pEC_{50} (M)</td>
</tr>
<tr>
<td>Control</td>
<td>2.76 ± 0.92</td>
<td>6.35 ± 0.13</td>
</tr>
<tr>
<td>Time control</td>
<td>2.87 ± 0.88</td>
<td>6.53 ± 0.20</td>
</tr>
<tr>
<td>Before L-NAME</td>
<td>1.77 ± 0.40</td>
<td>6.68 ± 0.34</td>
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<tr>
<td>Before EPO</td>
<td>4.15 ± 1.69</td>
<td>6.15 ± 0.15</td>
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<tr>
<td>+ EPO (0.1 unit·ml⁻¹)</td>
<td>3.70 ± 1.41</td>
<td>5.96 ± 0.11</td>
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<tr>
<td>Before EPO</td>
<td>2.17 ± 0.35</td>
<td>6.26 ± 0.06</td>
</tr>
<tr>
<td>+ EPO (20 units·ml⁻¹)</td>
<td>1.72 ± 0.28**</td>
<td>6.18 ± 0.10</td>
</tr>
<tr>
<td>+ L-NAME and before EPO</td>
<td>2.23 ± 0.43</td>
<td>5.82 ± 0.14</td>
</tr>
<tr>
<td>+ L-NAME and EPO (20 units·ml⁻¹)</td>
<td>1.54 ± 0.35**</td>
<td>5.58 ± 0.11**</td>
</tr>
</tbody>
</table>

Figure 1  Effects of EPO on noradrenaline-induced contractions
Additions were as follows: (A) 0.1 unit·ml⁻¹ EPO (n = 6); (B) 20 units·ml⁻¹ EPO (n = 7); (C) 20 units·ml⁻¹ EPO and 0.1 mM L-NAME (n = 6). ○, Before treatment; ■, after treatment. ***P < 0.001 compared with value before treatment. (D) This curve plots the difference in the degree of inhibition at each concentration of noradrenaline in the presence of 20 units·ml⁻¹ EPO alone (○) and that generated in the presence of EPO combined with L-NAME (■).

Noradrenaline induced concentration-dependent contractions of the vessels, with a maximum response at around 3–5 µM noradrenaline. Repetition of the concentration–response curve as a time control was not associated with a significant change in the response (Table 3). Incubation with L-NAME had no effect on the concentration–response relationship for noradrenaline (Table 3). Pre-incubation with EPO at 0.1 unit·ml⁻¹ appeared to attenuate the response to noradrenaline, although this did not reach statistical significance (Figure 1A; Table 3), and
Figure 2  Effects of EPO on KCl-induced contractions
Additions were as follows: (A) 0.1 unit·ml$^{-1}$ EPO ($n = 6$); (B) 20 units·ml$^{-1}$ EPO ($n = 7$); (C) 20 units·ml$^{-1}$ EPO and 0.1 mM L-NAME ($n = 6$). ◯, Before treatment; ■, after treatment. *** $P < 0.001$ compared with value before treatment. (D) This curve plots the difference in the degree of inhibition at each concentration of KCl in the presence of 20 units·ml$^{-1}$ EPO alone (◇) and that generated in the presence of EPO combined with i-NAME (■).

Figure 3  Effects of i-NAME on acetylcholine-induced relaxation
(A) Time control ($n = 11$); (B) effect of i-NAME ($n = 8$). ◯, Before treatment; ■, after treatment. *** $P < 0.001$ compared with value before treatment.

had no effect on either maximum tension or pEC$_{50}$. In contrast, EPO at 20 units·ml$^{-1}$ significantly suppressed the contractile response to noradrenaline ($P < 0.001$; Figure 1B), with a decrease in the maximum tension (by 21%; $P < 0.01$) (Table 3). The inhibitory effect of EPO (20 units·ml$^{-1}$) alone at each dose of noradrenaline was compared with the inhibition obtained in the presence of a combination of EPO and i-NAME over the noradrenaline concentration range (Figures 1C and 1D), and this showed that the action of EPO on noradrenaline-induced contraction was enhanced in the presence of i-NAME ($P < 0.01$; Figure 1D).

KCl induced a concentration-dependent contraction of the vessels, with a maximum response at around 125 mM; this response did not change when repeated as a time control (Table 3). Furthermore, incubation with i-NAME did not alter the concentration–response relationship for KCl (Table 3). In the presence of 0.1 unit·ml$^{-1}$ EPO, the concentration–response curve for KCl was displaced to the right (Figure 2A; Table 3), but this did not reach statistical significance. However, when the EPO concentration was increased to 20 units·ml$^{-1}$, there was a significant shift of the curve to the right, and the maximum response was reduced by 28% ($P < 0.001$).
and the pEC<sub>50</sub> value by 7% (P < 0.05) (Figure 2B; Table 3). In the presence of l-NAME, the suppression of the response to KCl by EPO was sustained (P < 0.001) (Figure 2C), giving a maximum suppression of 34% (P < 0.001) (Table 3) and a decrease in the pEC<sub>50</sub> value of 9% (P < 0.05) (Figure 2B; Table 3). The inhibitory effect of EPO on KCl-induced contraction was enhanced in the presence of l-NAME (P < 0.001) (Figure 2D; Table 3).

The cumulative administration of ET-1 induced concentration-dependent contractions of the vessels, with a maximum response of 1.98 ± 0.39 mN·mm<sup>-1</sup> at around 0.05 µM ET-1 and a pEC<sub>50</sub> of 8.74 ± 0.16 M. Incubation with EPO at 20 units·ml<sup>-1</sup> had no effect on the concentration–response relationship for ET-1 (maximum tension 1.73 ± 0.27 mN·mm<sup>-1</sup>; pEC<sub>50</sub> 8.75 ± 0.18 M).

Acetylcholine induced a concentration-dependent relaxation of the vessels, with a maximum response at around 5 µM acetylcholine, which was not altered in the time control with respect to either the magnitude or the pattern of the response obtained (Figure 3A; Table 4). Incubation of the vessels with l-NAME caused a marked suppression of acetylcholine-induced relaxation (P < 0.001) (Figure 3B), with the maximum response decreased by 41% (P < 0.001) (Table 4), although the pEC<sub>50</sub> was not affected (Table 4). EPO at either 0.1 or 20 units·ml<sup>-1</sup> had no effect on the size or shape of the acetylcholine-induced relaxation, either alone or in the presence of l-NAME (Table 4).

Bradykinin induced concentration-dependent relaxation of the vessels, with a maximum response at around 3 µM bradykinin, but these effects were variable. Therefore the vessels were discarded from the study if the initial relaxation was less than 40% of the maximum tension obtained in response to noradrenaline (Table 4). Incubation with l-NAME did not inhibit bradykinin-induced relaxation (Table 4), and pre-incubation with EPO (20 units·ml<sup>-1</sup>) had no effect on either the shape or the size of the bradykinin-induced relaxation response (Table 4).

Sodium nitroprusside induced concentration-dependent relaxation of the vessels, and this response was not affected by EPO at 0.1 unit·ml<sup>-1</sup> (in the absence and presence of EPO: maximum tension, 78.9 ± 5.96% and 79.9 ± 3.29% respectively; pEC<sub>50</sub> 5.92 ± 0.19 and 6.27 ± 0.33 M respectively).

**DISCUSSION**

The aim of this study was to examine the impact of EPO on the pharmacological properties of two types of human resistance vessels, i.e. subcutaneous arteries from patients with renal failure, in whom EPO is known to cause hypertension, presumably due in part to an action to raise tone in these vessels; and renal interlobular arteries from normal subjects, as these vessels, even in renal failure patients, appear to respond differently to EPO treatment. This was done by assessing whether acute application of EPO had any direct actions, and then determining whether EPO treatment modified the responsiveness to a number of vasoconstrictor and vasodilator compounds. Two concentrations of EPO were evaluated: a low concentration representative of that occurring in patients given the hormone therapeutically, and a high concentration which might reflect that occurring in the renal interstitium and therefore surrounding the renal interlobular arteries. It is important to recognize that the availability of human tissue is limited and, at times, the tissue may be collected under less than ideal conditions. One consequence of this is that there can be great

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**Table 4 Maximum responses and pEC<sub>50</sub> values for relaxation of human renal interlobular arteries**

For each protocol, n = 6–11 vessels derived from 2–5 subjects. *****P < 0.001 compared with value before pretreatment.

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<th>Acetylcholine</th>
<th>Bradykinin</th>
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<tr>
<td></td>
<td>Max. tension (mN·mm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>pEC&lt;sub&gt;50&lt;/sub&gt; (M)</td>
</tr>
<tr>
<td>Control</td>
<td>64.9 ± 5.51</td>
<td>6.04 ± 0.12</td>
</tr>
<tr>
<td>Time control</td>
<td>66.4 ± 7.22</td>
<td>6.59 ± 0.10</td>
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<tr>
<td>Before l-NAME</td>
<td>72.8 ± 5.80</td>
<td>7.02 ± 0.14</td>
</tr>
<tr>
<td>+ l-NAME</td>
<td>42.8 ± 4.97**</td>
<td>6.88 ± 0.07</td>
</tr>
<tr>
<td>Before EPO</td>
<td>75.2 ± 3.35</td>
<td>6.83 ± 0.05</td>
</tr>
<tr>
<td>+ EPO (0.1 unit·ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>79.4 ± 6.44</td>
<td>6.63 ± 0.08</td>
</tr>
<tr>
<td>Before EPO</td>
<td>73.2 ± 6.11</td>
<td>6.97 ± 0.10</td>
</tr>
<tr>
<td>+ EPO (20 units·ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>79.1 ± 5.62</td>
<td>6.75 ± 0.08</td>
</tr>
<tr>
<td>+ l-NAME and before EPO</td>
<td>35.7 ± 7.34</td>
<td>6.60 ± 0.20</td>
</tr>
<tr>
<td>+ l-NAME and EPO (20 units·ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>39.8 ± 9.59</td>
<td>6.40 ± 0.32</td>
</tr>
</tbody>
</table>
variability in the baseline characteristics of the vessels, their sensitivity to the different agents and the maximal tone developed. In an attempt to overcome some of these problems, all time control studies were performed rigorously.

Contraction
Noradrenaline and KCl induced concentration-dependent contractions of the vessels from the uraemic patients, and with both agonists repeated exposure led to a small reduction in the size of the response obtained. Interestingly, this time-related shift in responsiveness was similar to that reported for similar arteries from normal subjects [11], and may reflect an inherent instability of this type of human vessel. In contrast, 1-NAME actually caused a leftward shift in the relationships, causing enhancement of the noradrenaline- and KCl-induced contractions, suggesting that NO is one factor that might have contributed to this desensitization. In normal human subcutaneous arteries, Richards [11] found that the time-related decrease in the response to noradrenaline was prevented in the presence of Methylene Blue, which is equipotent or slightly more potent than 1-NAME in inhibition of the NO/cGMP pathway [12]. Together, these observations suggest that production of NO is enhanced in subcutaneous arteries from uraemic subjects, but this needs to be confirmed by a direct comparison, and the isoform of NO synthase (NOS) responsible needs to be investigated. Moreover, this effect of 1-NAME was observed irrespective of whether the contraction was induced via a receptor-mediated (noradrenaline) or voltage-dependent (potassium) mechanism. Thus it is possible to speculate that, in the study reported herein, undertaken over 3 h after the vessels had been removed from the uraemic plasma, the inhibitory effect of potential plasma factors on NOS would have been removed, with the consequence (see below) that NOS might be expressed at an enhanced level. This would help to explain the observation that blockade of NOS activity in the vessels from these patients in the present study resulted in a larger effect than with vessels from normal subjects [11]. This remains to be investigated.

With human renal interlobular arteries, both noradrenaline and KCl induced concentration-dependent contractions of the vessels over a concentration range similar to that seen for human subcutaneous arteries [13]. These responses were very repeatable, in contrast with the observations in human subcutaneous arteries [13], where maximum responses were very variable. The reasons underlying this variability in vasoconstrictor responses between human subcutaneous arteries and renal interlobular arteries may reflect the different functions of the organs from which the vasculatures were taken. In the presence of 1-NAME, the contractile responses of the renal interlobular arteries to noradrenaline and KCl were unchanged, suggesting that NO was not involved. These results are consistent with those obtained with human omental arteries [14], but different from those observed with subcutaneous arteries from normal subjects [11] and uraemic patients (the present study).

Relaxation
It is of interest that the maximum relaxation in response to acetylcholine of subcutaneous arteries (precontracted by noradrenaline) from uraemic subjects in the present study was similar to that observed for arteries from normotensive subjects [11,15]. These observations provide further support for the view that uraemia has no impact on the effectiveness of the intrinsic endothelium-dependent relaxation factor (EDRF) system in this particular vascular bed. The inhibition by 1-NAME of the acetylcholine-induced relaxation of the vessels from uraemic patients was similar in degree and magnitude to that observed with vessels from normotensive subjects using Methylene Blue [11]. The similarity in the degree of inhibition caused by the NO inhibitors of the acetylcholine-induced relaxation of the same-sized vessels obtained from uraemic patients and normal subjects could be interpreted as indicating that the activity of the endothelial NOS isoform was not blunted in the uraemic patients. The fact that the maximum relaxation induced by bradykinin was similar to that obtained on acetylcholine-induced relaxation of the same vessels also supports the hypothesis that, under uraemic conditions, the EDRF system was relatively normal in this vascular bed.

Acetylcholine produced a concentration-dependent relaxation of the human renal interlobular arteries, the magnitude of which was similar to that obtained with human subcutaneous arteries but much larger than that reported with rat renal arcuate arteries [16]. Because the degree of acetylcholine-induced relaxation was much smaller in the rat than in the human intrarenal arteries, the possibility arises that the underlying mechanisms might be different, and that NO might not be the only factor involved. It was evident that, in the present study, bradykinin-induced relaxation of the renal interlobular arteries was not dependent on NO, as 1-NAME was without effect. The reason for this is unclear, but it is recognized that bradykinin-induced vasodilatation can be mediated via B1-bradykinin receptors, which lead to NO release from the endothelium, whereas B2 receptors cause the release of vasodilator prostaglandins or the opening of K+-ATP channels [17]. Therefore the lack of inhibition of bradykinin-induced relaxation of human renal interlobular arteries by 1-NAME could be due to a dominant role of B1 receptors in this tissue.

Effects of EPO
The findings with EPO were quite clear-cut, in that the hormone had no effect on the resting tension of either subcutaneous or renal interlobular arteries. Moreover, it
had minimal effects on the responses of subcutaneous arteries from uraemic subjects to a number of agonists. Importantly, using an alternative approach of examining the interaction with other agonists, the high dose of EPO attenuated the response of the human renal interlobular arteries to noradrenaline and KCl. These were unexpected findings, and in direct contrast with the effects observed in human subcutaneous arteries from uraemic subjects, rabbit aorta and carotid arteries [18], where EPO was without effect. Moreover, in human renal interlobular vessels, L-NAME enhanced the EPO-induced inhibition of vasoconstriction, suggesting that a rather complicated interaction exists which has yet to be explored. Evidently, this interaction does not extend to the dilation of vessels, as EPO did not affect the relaxation induced by either acetylcholine or bradykinin, the major stimulators of EDRF release. Only in human renal interlobular arteries were contractions induced by noradrenaline and KCl suppressed by EPO, which might indicate a species and vascular bed specificity. The kidney is the major site for the production of EPO in adults, and the possibility arises that there might be more EPO receptors on the vasculature of this organ, which may have a role in the modulating production of EPO.

Conclusions
Studies using human subcutaneous arteries from uraemic subjects have demonstrated that noradrenaline- and KCl-induced vasoconstrictions were enhanced following incubation with L-NAME. Moreover, NO appeared to only partially mediate the acetylcholine- and bradykinin-induced relaxation of subcutaneous arteries from uraemic subjects and the acetylcholine-induced relaxation of renal interlobular arteries. Noradrenaline- and KCl-induced contractions of human renal interlobular arteries were unaffected by NO, but were attenuated by EPO, and this latter effect was enhanced in the presence of L-NAME. EPO did not affect the resting tension of either vessel type, and had a relatively small effect on the vasoactive responses in subcutaneous arteries from uraemic subjects.

REFERENCES

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