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Impaired endothelial function in isolated human uremic resistance arteries

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Background. Patients with chronic renal failure (CRF) face a markedly increased risk of cardiovascular death. CRF is frequently complicated by hypertension and changes in both the heart (left ventricular hypertrophy) and the vasculature (endothelial dysfunction and accelerated atherosclerosis). The mechanisms underlying changes in vascular function and specifically endothelial dysfunction are unclear. This present study therefore examined subcutaneous resistance artery function in vitro, comparing adult uremic patients and controls using wire myography.

Methods. Subcutaneous fat biopsies were obtained from 12 patients with CRF (median serum creatinine 735 μmol/L) at the time of renal transplantation or peritoneal dialysis catheter insertion, and from eight controls without renal disease at the time of abdominal surgery. Resistance arteries were mounted on a wire myograph. Their contractile ability was tested with high potassium depolarization, and endothelial integrity was tested by relaxation to acetylcholine. Cumulative concentration-response curves were then constructed for norepinephrine, endothelin-1, acetylcholine, and sodium nitroprusside (SNP).

Results. Following preconstriction with norepinephrine, vessels from uremic patients vasodilated less well to acetylcholine compared with vessels from controls [maximum % relaxation 77% (range 41, 97) vs. 98% (78, 100), $P < 0.001$]. The vasodilation to SNP was similar [95% (63, 100) vs. 94% (71, 100), $P = 0.751$]. There was a trend toward increased maximum pressure (kPa) achieved with both norepinephrine and endothelin-1 in vessels from uremic patients, and the contractions to both of these agents were more prolonged in the uremic vessels.

Conclusions. The pattern of normal vasodilation to SNP but reduced vasodilation to acetylcholine is consistent with endothelial dysfunction due to impaired nitric oxide (NO) production in uremic vessels. Similar results have been demonstrated in vivo in uremia, one suggested mechanism being accumulation of endogenous inhibitors of NO synthase such as asymmetric dimethylarginine (ADMA). This in vitro study suggests that a short-lived circulating factor is not entirely responsible and that there may be an inherent abnormality in endothelial function in uremia, although the exact pathophysiology remains unclear. Endothelial dysfunction may predispose the patient to accelerated atherosclerosis and may be involved in the pathogenesis of hypertension in end-stage renal failure.

Key words: uremia, vascular endothelium, nitric oxide, wire myography, atherosclerosis, hypertension, ESRD.

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Patients with advanced renal failure no longer die of uremia but are successfully treated by dialysis and renal transplantation, with constantly improving long-term results. The new threat for patients on renal replacement therapy is premature cardiovascular death (CVD), with studies suggesting a 10- to 20-fold increased risk of CVD compared with the general population [1]. The mechanisms underlying this greatly increased risk of CVD are likely to involve changes in both the heart and the blood vessels. Uremia is known to be associated with hypertension and left ventricular hypertrophy (LVH) [2], which may predispose to sudden arrhythmic death. Changes also occur in the blood vessels in uremia, including vascular hypertrophy [3], calcification [4], and premature atherosclerosis [5], the clinical consequences being peripheral vascular disease, cerebrovascular disease, and ischemic heart disease [6].

The vascular endothelium plays a crucial role in the maintenance of blood vessel tone and in the prevention of atherosclerosis [7]. Endothelial cells produce and release vasoactive substances, including the vasoconstrictors endothelin and thromboxane A_2, and the vasodilator substances nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin. Endothelial dysfunction is an early abnormality in the development of atherosclerotic plaques and is present in hyperlipidemia [8], diabetes [9], hypertension [10], and cigarette smokers [11]. In uremia, several in vivo studies have also suggested endothelial dysfunction and, in particular, a reduction in the production or effect of NO [12–14]. One proposed mechanism is that there is an accumulation of endogenous inhibitors of NO synthase (NOS) such as asymmetric dimethylarginine (ADMA) in chronic renal failure (CRF) [15]. Our present study sought to examine endothelial function in uremia and to determine the rele-

Table 1. Background characteristics for uremic patients and controls expressed as median (range) with comparison by chi-square or Mann-Whitney U tests

	Control ($N = 8$)	Uremic ($N = 12$)	P value
Male	5	8	NS
Smoker	3	4	NS
Age *years*	42 (31, 73)	46 (20, 77)	0.910
Urea *mmol/L*	5.5 (2.7, 10.2)	21.8 (7.2, 35.1)	<0.001
Creatinine *μmol/L*	85 (66, 123)	735 (476, 1041)	<0.001
Glucose *mmol/L*	4.7 (4.3, 6.0)	5.2 (3.4, 6.8)	0.913
Cholesterol *mmol/L*	4.6 (2.8, 7.4)	4.5 (3.7, 5.3)	0.408
Triglyceride *mmol/L*	0.9 (0.7, 3.2)	1.4 (0.8, 2.0)	0.902
Hemoglobin *g/dL*	13.2 (11.8, 14.5)	9.8 (7.9, 11.1)	<0.001
SBP *mm Hg*	124 (106, 152)	149 (112, 180)	0.043
DBP *mm Hg*	78 (68, 88)	82.5 (52, 110)	0.601
BMI	23.6 (16.8, 28.3)	25.2 (19.8, 30.8)	0.315

Abbreviations are: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

vance of circulating inhibitors of NOS to endothelial dysfunction by studying resistance vessels removed from the circulation. Using the in vitro technique of wire myography, we examined the effects of endothelium-dependent (acetylcholine) and independent [sodium nitroprusside (SNP)] vasodilators and the effects of the vasoconstrictors norepinephrine and endothelin-1.

METHODS

Materials

Acetylcholine, SNP, and norepinephrine were purchased from Sigma-Aldrich (Poole, Dorset, UK). Human endothelin-1 was purchased from ICN (Thame, UK). Experiments were performed on a Mulvany-Halpern 4-channel myograph (JP Trading, Aarhus, Denmark).

Human subcutaneous resistance arteries

Twelve patients with CRF agreed to have a subcutaneous fat biopsy at the time of continuous ambulatory peritoneal dialysis (CAPD) catheter insertion or renal transplantation under general anesthetic. Control tissue was obtained from volunteers without known renal disease who were undergoing abdominal surgery or hernia repair. All subjects gave written informed consent, and the protocol was approved by the local ethics committee. The background characteristics of patients and controls are detailed in Table 1. Subjects were excluded if they had known cardiovascular disease [angina pectoris, previous myocardial infarction or coronary artery bypass grafting (CABG)], diabetes, or a history of vasculitis.

At the time of operation, an ellipse of skin approximately 2 × 1 cm with adherent fat was removed from the anterior abdominal wall to a depth of 2 cm. The tissue was immediately placed into chilled 0.9% saline and transported to the laboratory where it was placed in cold physiological salt solution [PSS; composition in mmol/L: NaCl 118.4, KCl 4.7, $MgSO_4 \cdot H_2O$ 1.2, $NaHCO_3$ 24.9, $CaCl_2$ 2.5, glucose 11.1, and ethylenediaminetetraacetic acid (EDTA) 0.023, which gives a pH of 7.4 when aerated with a 5% CO_2/95% O_2 mixture]. Where possible, four resistance vessels ~2 mm long were dissected and mounted on two 40 μm diameter stainless steel wires in a four-channel myograph. The temperature was maintained at 37°C, and the medium was continuously aerated with a 5% CO_2/95% O_2 mixture.

Protocol

After a 30-minute acclimatization period, the vessel was normalized at internal diameter L_0 [16]; the arteries were then exposed twice to KPSS (PSS with KCl substituted for NaCl on an equimolar basis) and once to norepinephrine 10 μmol/L. After a plateau contraction had been reached to norepinephrine, acetylcholine 3 μmol/L was added to assess endothelium-dependent vasodilation and thus the presence of an intact endothelium. Vessels that failed to contract to KPSS or norepinephrine or that failed to relax >50% to acetylcholine were discarded (10 in the control group and 12 in the uremic group).

Two separate protocols were then carried out. A cumulative concentration-response curve to norepinephrine (10^{-9} mol/L to 3×10^{-4} mol/L) was constructed with two of the vessels involving exposure to each dose of norepinephrine for four minutes or until a peak response at that concentration was reached. Following a washout period of 30 minutes and re-establishment of baseline, a cumulative concentration-response curve to endothelin-1 (10^{-12} mol/L to 3×10^{-6} mol/L) was constructed, with arteries exposed to each concentration of endothelin-1 for 10 minutes or until a peak was reached.

With the two remaining vessels, after preconstriction with norepinephrine (3×10^{-6} mol/L), a concentration response curve to acetylcholine (10^{-9} to 3×10^{-4} mol/L) was constructed involving exposure to each concentration of acetylcholine for two minutes or until the maximum relaxation was achieved. Again, following a washout period of 30 minutes, the arteries were preconstricted with norepinephrine (3×10^{-6} mol/L), and a concentration-response curve was performed for SNP (10^{-9} to 3×10^{-4} mol/L), with arteries being in contact with each concentration of SNP for two minutes or until a plateau had been reached.

When a complete concentration-response curve was not possible for technical reasons, the vessel was discarded. The number of vessels studied for each drug and the corresponding size of these vessels are detailed in Table 2.

Statistical analysis

Contractile responses were expressed as an increase in active effective pressure (kPa), calculated as an increase in isometric tension above resting divided by the normal-

Table 2. Background data for vessels

	Control	Uremic	*P* value
Norepinephrine			
Number	17	22	
L_0	395.8 (212.2, 500.9)	360.9 (241.2, 536.4)	0.377
Endothelin-1			
Number	14	16	
L_0	404.5 (240.2, 500.9)	374.6 (262.8, 530.5)	0.697
Acetylcholine			
Number	19	23	
L_0	413.3 (212.2, 500.9)	345.6 (183.2, 536.4)	0.114
Sodium nitroprusside			
Number	12	20	
L_0	358.6 (212.2, 474.4)	363.7 (183.2, 536.4)	0.833

Number is the number of vessels in which a complete concentration-response curve was obtained for that particular drug, and L_0 is the normalized internal diameter of the vessels. Comparison is by the Mann-Whitney U test.

Table 3. Potency [EC_{50} (molar)] of noradrenaline, endothelin-1, acetylcholine, and sodium nitroprusside in uremic and control vessels

	Control	Uremic	*P* value
NA EC_{50}	2.23×10^{-7} (0.72×10^{-7}, 21.30×10^{-7})	6.78×10^{-7} (0.23×10^{-7}, 58.10×10^{-7})	0.163
ET-1 EC_{50}	6.01×10^{-10} (0.15×10^{-10}, 13.50×10^{-10})	9.50×10^{-10} (2.69×10^{-10}, 71.04×10^{-10})	0.208
ACh EC_{50}	4.81×10^{-8} (0.69×10^{-8}, 59.51×10^{-8})	7.35×10^{-8} (0.74×10^{-8}, 37.73×10^{-8})	0.604
SNP EC_{50}	5.40×10^{-8} (0.58×10^{-8}, 87.12×10^{-8})	5.43×10^{-8} (0.40×10^{-8}, 19.05×10^{-8})	0.716
Ach max % relaxation	98 (78, 100)	77 (41, 97)	<0.001
SNP max % relaxation	94 (71, 100)	95 (63, 100)	0.751

Data are expressed as median (range) with comparison by Mann-Whitney U test. Abbreviations are: NA, norepinephrine; ET-1, endothelin-1; ACh, acetylcholine; SNP, sodium nitroprusside. Maximum % relaxation from baseline of acetylcholine and sodium nitroprusside is expressed as median (range).

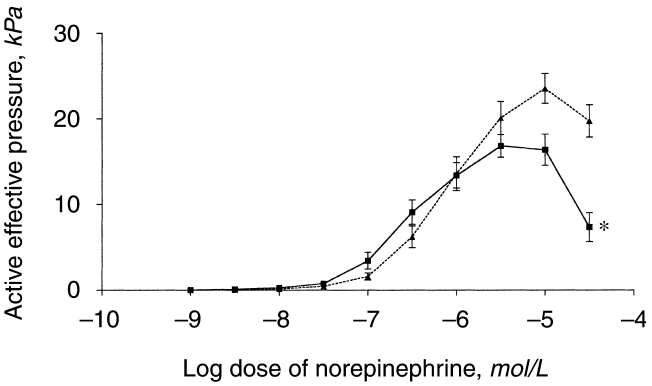

Fig. 1. Concentration-response curves for norepinephrine in uremic (▲) and control (■) groups. Data are mean ± SEM; *$P = 0.001$.

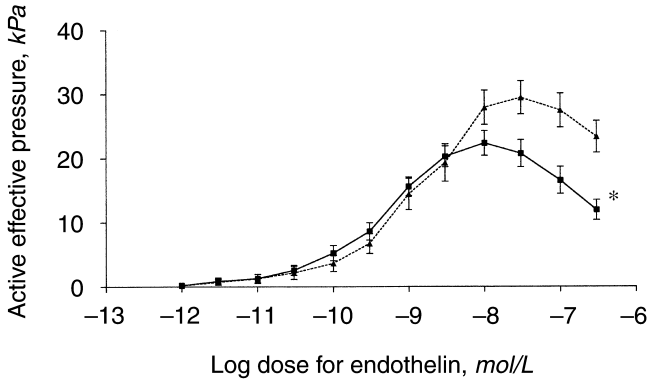

Fig. 2. Concentration-response curves for endothelin-1 in uremic (▲) and control (■) groups. Data are mean ± SEM; *$P = 0.01$.

ized internal radius. Agonist potency was expressed in terms of maximum response obtained, and EC_{50} value, this being the concentration required to produce 50% of the maximum response. For SNP and acetylcholine, the response was expressed as maximum % relaxation from baseline. A comparison between groups was by Mann-Whitney U test. All analyses were performed using SPSS for Windows (version 7.5), with significance at a level of $P < 0.05$.

RESULTS

Patients and controls were of a similar age and sex distribution and had similar total serum cholesterols and smoking habits (Table 1). Hemoglobin was significantly lower in the uremic patients, and systolic blood pressure was significantly higher, as expected. Table 2 details the number and size of the vessels used for each drug. There was no significant difference in vessel size between groups.

The results for the vasoconstrictors norepinephrine and endothelin-1 are shown in Table 3 and in Figures 1 and 2. The potency of norepinephrine and endothelin-1 were similar in uremic and control groups (no significant difference in EC_{50}). However, from the concentration response curves, it can be seen that there was a tendency for a more sustained contraction to these agents with the response (kPa) at the highest dose of norepinephrine

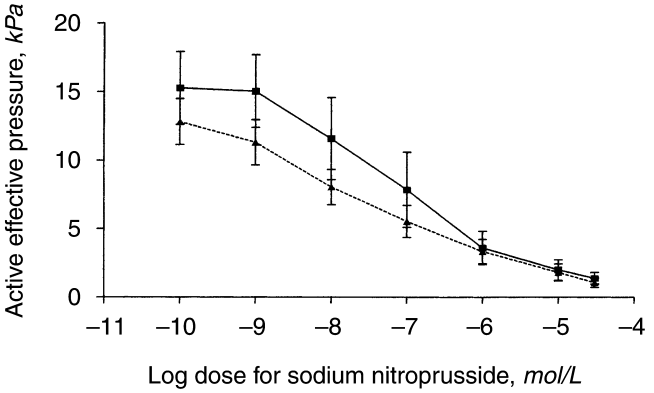

Fig. 3. Concentration-response curves for sodium nitroprusside in uremic (▲) and control (■) groups. Data are mean ± SEM.

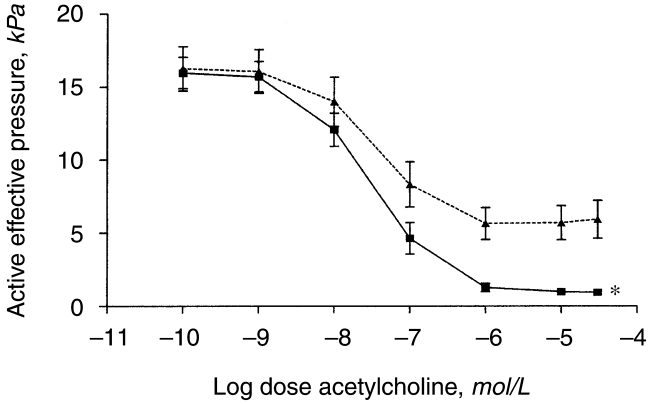

Fig. 4. Concentration-response curves for acetylcholine in uremic (▲) and control (■) groups. Data are mean ± SEM; *$P < 0.001$.

being significantly greater in the uremic group [16.89 (1.7, 30.6) uremic vs. 6.96 (0, 25.9) control, $P = 0.001$] and the response to the highest dose of endothelin-1 being significantly greater in the uremic group [19.08 (8.9, 37.8) uremic vs. 13.44 (2.2, 22.2) control, $P = 0.006$].

The results for the endothelium-independent vasodilator SNP are detailed in Table 3 and Figure 3. Again, the potency of this agent was similar in uremic and control groups, and the same maximum relaxation was obtained in the two groups. However, when the results were examined for the endothelium-dependent vasodilator acetylcholine (Table 3 and Fig. 4), the maximum relaxation obtained in uremic patients (77%, range 41, 97) was significantly lower than that obtained in controls (98%, range 78, 100, $P < 0.001$).

DISCUSSION

The healthy vascular endothelium acts to prevent the development of atherosclerosis principally through the production and release of NO. NO is produced continuously under resting conditions from L-arginine and contributes to basal vessel tone. NO, in addition to vasodilating, inhibits platelet aggregation, inhibits vascular smooth muscle proliferation, and inhibits monocyte adhesion and migration. Dysfunction of the vascular endothelium is likely to manifest in several ways, but the most commonly studied defect is a reduction in endothelium-dependent vasodilation, mainly as a result of reduced NO production and/or release. Such a defect has been demonstrated in several conditions, including hypercholesterolemia [8], diabetes mellitus [9], and in cigarette smokers [11]. In our study, subjects and controls were matched as closely as possible for these variables. In hypertension, most studies suggest impaired endothelium-dependent vasodilation [10, 17], although Cockcroft et al [18] found that endothelium-dependent vasodilation was preserved. In our study, systolic blood pressure (but not diastolic) was higher in uremic subjects compared with controls.

As chronic renal failure is associated with accelerated atherosclerosis, many have proposed that there is likely to be a circulating "uremic factor" responsible for these changes. The vascular endothelium would seem a reasonable target for such a uremic factor. Cell culture studies have suggested that uremic serum may inhibit NO synthase (iNOS) [19] and that uremic serum adversely affects endothelial extracellular matrix production and attachment of endothelial cells to the subendothelium [20]. Noninvasive studies using Doppler ultrasound measurement of radial or brachial artery diameter or dorsal hand vein diameter have revealed impaired endothelium-dependent vasodilation in adult hemodialysis patients [12] and in uremic children not yet requiring dialysis [13]. We have also shown in adult patients with CRF that endothelium-dependent vasodilation was attenuated when studied using forearm plethysmography [14].

In this present study, using an in vitro technique, we have again demonstrated impaired endothelium-dependent vasodilation and preserved endothelium-independent vasodilation in uremia. Thus, there is general agreement that uremic individuals have endothelial dysfunction, but the exact mechanisms involved are unclear. While it is possible that endothelial dysfunction simply reflects the synergistic effect of multiple "traditional" cardiovascular risk factors, the magnitude of the increased risk in uremics over diabetics (who also have several risk factors for CVD) suggests that there may indeed be a "uremic factor(s)."

Potential candidates for an atherogenic "uremic factor" include homocysteine [21], increased oxidative stress [22], and endogenous inhibitors of NO synthase such as ADMA and N^G-monomethyl-L-arginine (L-NMMA) [15], two naturally occurring substances that accumulate in renal failure because of reduced excretion and/or me-

tabolism. An eightfold rise in plasma ADMA concentrations has been reported in uremic patients compared with healthy controls [15], with levels approaching those required to raise blood pressure in experimental animals. Other groups have subsequently demonstrated smaller increases in ADMA concentrations in CRF [23, 24], and it remains to be seen whether the levels reached are physiologically significant.

This study shows that there is endothelial dysfunction in human uremic vessels in vitro, in the absence of circulating factors such as ADMA or homocysteine. This suggests that the defect in endothelial function is neither readily reversible nor caused by a short-lived circulating factor. ADMA may still play a role, however, as a recent study has demonstrated that ADMA accumulates intracellularly, and therefore may have longer lasting effects [25]. Our results are in direct contrast to those of a recently published study that found normal endothelium-dependent vasodilation in resistance vessels from uremic rats [26], although the vessels differed in being from the mesentery. Other potential explanations include species differences, that the duration of uremia in the animal model was too short, or the fact that the rats were pretreated with cyclooxygenase inhibitors.

A potential confounding factor in our study was the higher systolic blood pressure in the patients compared with controls. Several studies in hypertension have demonstrated endothelial dysfunction [10, 17], although others have not [18], and we cannot therefore conclude that the results of our current study are unique to uremia.

The results for norepinephrine are in keeping with those of an earlier myography study [16]. Although we did not demonstrate any difference in the EC_{50} for norepinephrine or endothelin-1, the concentration-response curves suggest that the uremic patients had a greater and more prolonged response to both of these vasoconstrictors. This may reflect a reduced counterbalancing effect of NO release or be secondary to changes in the arterial wall structure (vascular smooth muscle hypertrophy) in uremia.

In conclusion, this study demonstrates impaired endothelium-dependent vasodilation with preserved endothelium-independent vasodilation in uremic resistance arteries in vitro. The results support in vivo studies in renal failure, but contrast with those of the only other published myography study in uremia looking specifically at endothelial function [26]. Our results suggest that endothelial dysfunction, and in particular impaired endothelial NO production and/or release, is a defect that may predispose these patients to accelerated atherosclerosis. The underlying pathophysiology remains unclear.

It is likely that endothelial dysfunction develops in the earliest stages of progressive renal disease and that clinical studies are required to determine ways of preventing or reversing endothelial dysfunction. Several drugs, such as statins [8], angiotensin-converting enzyme inhibitors [27], vitamin C [28], folic acid [29], and vitamin E [30], are already known to improve endothelial function in other groups. Whether similar therapeutic measures will also improve endothelial function in uremic patients and reduce the excessive cardiovascular morbidity of this group has yet to be determined.

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