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Reduced endogenous endothelin-1–mediated vascular tone in chronic renal failure

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Reduced endogenous endothelin-1–mediated vascular tone in chronic renal failure.

Background. Endothelin-1 generated by the vascular endothelium contributes to basal vascular tone and blood pressure in healthy humans. Plasma concentrations of endothelin-1, which are elevated in chronic renal failure (CRF), may contribute to increased vascular tone.

Methods. We investigated the contribution of endogenous and exogenous endothelin-1 to the maintenance of vascular tone in patients with CRF (creatinine ≥ 200 μmol/liter) and in age- and sex-matched healthy subjects. In a series of experiments, we measured forearm vascular responses to intra-arterial norepinephrine (30 to 240 pmol/min), endothelin-1 (5 pmol/min), the selective endothelin A (ET_A) receptor antagonist BQ-123 (3 mg/hr), the mixed endothelin-converting enzyme and neutral endopeptidase inhibitor phosphoramidon (30 nmol/min), and the selective neutral endopeptidase inhibitor thiorphan (30 nmol/min).

Results. The maximum reduction in forearm blood flow (FBF) to norepinephrine in CRF (33 ± 7%) was similar to that in controls (43 ± 7%, $P = 0.53$). Endothelin-1 also produced a similar reduction in FBF in CRF (35 ± 6%) and controls (36 ± 5%, $P = 0.81$). BQ-123 increased FBF in CRF (11 ± 4%) but significantly less than in controls (44 ± 10%, $P = 0.02$). Phosphoramidon increased FBF in CRF (68 ± 20%), again significantly less than in controls (181 ± 41%, $P = 0.001$). Thiorphan reduced FBF similarly in CRF (22 ± 6%) and controls (14 ± 6%, $P = 0.39$). Responses to phosphoramidon were substantially greater than to BQ-123.

Conclusions. These studies show that endogenous generation of endothelin-1 contributes to the maintenance of resting vascular tone in patients with CRF, as well as in healthy subjects. Although the contribution of endogenous endothelin-1 to resting vascular tone appears to be reduced in CRF, ET_A receptor antagonism, and particularly endothelin-converting enzyme inhibition, should be explored as means by which to reduce vascular tone and blood pressure in patients with CRF.

Key words: endothelin receptors, BQ-123, blood pressure, vasoconstriction, hypertension, renal failure.

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Endothelin-1 is an endothelium-derived 21-amino acid peptide with sustained vasoconstrictor properties [1]. Endothelin-1 is generated from an inactive 38-amino acid precursor, big endothelin-1, through the action of endothelin-converting enzyme (ECE), a unique membrane-bound neutral metalloprotease [2]. Endothelin-1 binds to at least two receptors [3]: The endothelin A (ET_A) receptor is present on vascular smooth muscle cells in which it plays a major role in causing vasoconstriction [4]; the ET_B receptor is present on endothelial cells in which it mediates release of endothelium-derived vasodilator substances [5], and also on vascular smooth muscle cells in which it mediates vasoconstriction [6]. Endogenous generation of endothelin-1 has been shown to contribute to the maintenance of basal vascular tone [4] and blood pressure [7] in healthy humans.

Plasma concentrations of immunoreactive big endothelin-1 and endothelin-1 are elevated in renal failure [8, 9]. Because these high plasma endothelin concentrations may be within a range sufficient to cause vasoconstriction [10], plasma endothelin-1 may play a role in the development, or maintenance, of hypertension in chronic renal failure (CRF). Indeed, plasma endothelin concentrations are positively correlated with blood pressure in hemodialysis patients [11]. This contrasts to findings in patients with mild to moderate essential hypertension in whom no such relationship was found [12]. However, it is not known whether endothelin-1 contributes to elevated basal resistance vessel tone in patients with CRF.

Therefore, we have investigated the responses of forearm resistance vessels to brachial artery infusion of endothelin-1 and a control constrictor, norepinephrine, in patients with stable CRF who were independent of dialysis and in healthy age- and sex-matched control subjects. We also examined the contribution of endothelin-1 acting via the ET_A receptor to basal vascular tone by brachial artery infusion of a maximally effective dose [13, 14] of the specific ET_A receptor antagonist, BQ-123 [4, 15]. We further examined the contribution of endogenous generation of endothelin-1 to the maintenance of forearm vas-

cular tone by comparing responses to brachial artery infusion of the combined ECE and neutral endopeptidase (NEP) inhibitor, phosphoramidon [4, 16, 17], with those to the selective NEP inhibitor, thiorphan [4, 17].

METHODS

Subjects

A total of 11 patients (10 male, 1 female) with established chronic renal disease (creatinine ≥ 200 μmol/liter and stable blood pressure for 6 months or more) caused by IgA nephropathy ($N = 3$), obstructive uropathy ($N = 2$), reflux nephropathy ($N = 2$), nephrosclerosis ($N = 2$), interstitial nephritis ($N = 1$), and Alport's syndrome ($N = 1$) were recruited. All patients were independent of dialysis. Three subjects underwent all of the study protocols. Five subjects underwent the protocols involving infusion of norepinephrine, endothelin-1, and BQ-123 only, and three subjects underwent infusion of phosphoramidon and thiorphan only. If patients were taking antihypertensive or other vasoactive medication, this was withdrawn at least one week before each study. Ten age- and sex-matched healthy control subjects (nine male, one female) who were not taking any vasoactive medication were also recruited, of whom four undertook all the study protocols, and four the protocols involving infusion of norepinephrine, endothelin-1, and BQ-123 only. Two subjects underwent infusion of phosphoramidon and thiorphan only. All studies were conducted with the approval of the local ethics review committee, and all subjects gave their written informed consent to participate. Subjects avoided nonsteroidal anti-inflammatory drugs for the week before and caffeine-containing drinks or cigarettes on the day of each study. In addition, subjects fasted for at least three hours before each study.

Procedures

A 21 SWG cannula was inserted into a vein on the dominant arm under local anesthesia using 1% lidocaine hydrochloride (Lidocaine; Astra Pharmaceuticals Ltd., Kings Langley, UK) for the purpose of sampling blood. A 27 SWG steel cannula (Cooper's Needle Works, Birmingham, UK) was inserted into the brachial artery of the nondominant arm, under local anesthesia, for the purpose of intra-arterial infusion of drugs. Forearm blood flow (FBF) was measured in both arms using venous occlusion plethysmography [18] adapted for use with indium-and-gallium-in-silastic strain gauges [4]. Measurements were made for 10 seconds in every 15 seconds over a three-minute period. Blood flow to the hands was excluded during each three-minute measurement period.

Arterial pressure was measured using a well-validated semiautomated oscillometric method (Takeda UA 751; Takeda, Tokyo, Japan) [19, 20].

Drugs

Norepinephrine (30, 60, 120, and 240 pmol/min; Sanofi Winthrop Ltd., Guildford, UK), endothelin-1 (5 pmol/min; Clinalfa, Laufelfingen, Switzerland), BQ-123 (3 mg/hr; American Peptide Company, Sunnyvale, CA, USA), phosphoramidon (30 nmol/min; Clinalfa), thiorphan (30 nmol/min; Sigma Chemicals, Poole Dorset, UK), and physiologic saline (0.9%; Baxter Healthcare Ltd., Thetford, UK) were administered at locally but not systemically active doses via the brachial artery cannula. These doses were based on previous studies [4, 21]. All solutions were made up in physiologic saline. The norepinephrine solutions were made up in physiologic saline containing 100 μg/ml ascorbic acid (Evans Medical Ltd., Dunstable, UK) to avoid oxidation. The total rate of intra-arterial infusion was kept constant at 1 ml/min.

Design

Studies were performed in a quiet room maintained at a constant temperature of between 22°C and 25°C. After brachial artery and venous cannulation, saline was infused via the brachial artery cannula for 30 minutes, and blood was withdrawn from the venous cannula to measure plasma creatinine, endothelin-1, big endothelin-1, and hemoglobin. In eight patients with CRF and in eight control subjects, norepinephrine was then infused at incremental doses (30, 60, 120, and 240 pmol/min, each dose for 6 min) via the brachial artery cannula. After infusion of norepinephrine, saline was infused for 20 minutes to allow for the reversal of vasoconstriction. Endothelin-1 was then infused at a single dose of 5 pmol/min for one hour. FBF was recorded at 10-minute intervals during infusion of saline and during the last three minutes of each increment of the norepinephrine infusion and at 10-minute intervals during the endothelin-1 infusion. On a separate day, after the 30-minute infusion of saline, BQ-123 was infused in these subjects at a single dose of 3 mg/hr for one hour, and FBF was measured at five-minute intervals. In a further subgroup of three CRF patients and seven control subjects, the BQ-123 was infused at 3 mg/hr and was continued for two hours.

In a separate study in six patients with CRF and in six control subjects, after a 30-minute infusion of saline, phosphoramidon or thiorphan was infused, on separate days, at a single dose of 30 nmol/min for 90 minutes, and FBF was measured at 10-minute intervals.

In all studies, the mean of the final five blood flow measurements of each recording period was used for analysis. Arterial pressure was measured in the noninfused arm immediately after each blood flow recording. In each subject, infusions were on separate days, at least one week apart. The order in which the experiments were undertaken was randomized. However, vasoconstriction to endothelin-1 is sustained, whereas that to

norepinephrine is short lasting. Therefore, in the combined norepinephrine/endothelin-1 study, it was necessary to give norepinephrine first rather than randomize the order of infusions.

Analytical

Venous plasma samples for radioimmunoassay of endothelin-1 and big endothelin-1 were separated within 10 minutes and were stored at −80°C. Plasma biochemistry was measured using a Kodak Ectachem System E700 XRC analyzer (Kodak Diagnostics Ltd., Hemel Hempstead, Herts., UK) in the Department of Clinical Chemistry, Western General Hospital (Edinburgh, UK). Hemoglobin was measured in the Department of Hematology, Western General Hospital, using a Coulter STKS analyzer (Coulter Electronics Ltd., Luton, Beds., UK).

Plasma immunoreactive endothelin-1 and big endothelin-1 concentrations were measured using an acetic acid extraction technique [22] and a modified commercial radioimmunoassay using rabbit antihuman endothelin-1 or big endothelin-1 (Peninsula Laboratories Europe, St. Helens, UK) [23]. Briefly, sample extract was incubated with either endothelin-1 or big endothelin-1 antibody for 24 hours at 4°C. Following incubation, ^{125}I-labeled endothelin-1 (NEN Life Science Products, Boston, MA, USA) or big endothelin-1 (Peninsula Laboratories Europe) was added, and incubation was continued for an additional 20 hours at 4°C. Complexes were precipitated with Amerlex™ donkey antirabbit antibody (Amersham Life Sciences Ltd., Little Chalfont, Bucks., UK) and were counted for radioactivity. All endothelin values were expressed as picograms per milliliter. Recovery of endothelin-1 and big endothelin-1 was 89% and 91%, respectively. Intraassay and interassay coefficients of variations in our laboratory are 6.3% and 7.2%, respectively; the sensitivity of the assay for endothelin-1 was 0.25 pg/ml, and for big endothelin-1, sensitivity was 1 pg/ml.

Cross-reactivities of the endothelin-1 assay were endothelin-1 (100%), endothelin-2 (0%), endothelin-3 (0%), and big endothelin-1 (10%). The cross-reactivities of the big endothelin-1 assay were endothelin-1 (0%), endothelin-2 (0%), endothelin-3 (0%), and big endothelin-1 (100%).

Data presentation and statistics

Mean arterial pressure was calculated as diastolic arterial pressure plus one-third pulse pressure. FBF was calculated in milliliters per 100 ml of forearm tissue per minute. The ratio of FBF in the infused arm compared with that in the control arm was calculated for each measurement period. The ratio of FBF (infused:control arm) was measured in response to drugs and was expressed as a percentage of the ratio (infused:control arm) measured during the control period [24]. This method uses the noninfused arm as a contemporaneous control and compensates for the continual small adjustments affecting the circulation of both arms that occur even at rest and for other external systemic factors such as the level of arousal that may otherwise act as confounding factors [24].

All results are expressed as mean ± SEM. Dose–response relationships were compared using analysis of variance. All other parameters were analyzed by Student's paired or unpaired *t*-test as appropriate. Statistical analysis was performed using the STATVIEW 512+™ software (Brain-power Inc., Calabasas, CA, USA) for the Apple Macintosh microcomputer. Values of P of less than 0.05 were considered statistically significant.

RESULTS

There was no significant difference in age, sex, hemoglobin, or resting FBF between the patients with CRF and the healthy control subjects when receiving norepinephrine, endothelin-1, or BQ-123 (Tables 1 and 2). However, resting FBF was higher in patients with CRF who underwent the phosphoramidon/thiorphan studies compared with control subjects (Table 2). FBF in the noninfused arm did not change during any infusion period apart from when endothelin-1 and thiorphan was infused in control subjects (Table 2) when the FBF in the control arm increased, whereas the blood flow in the infused forearm decreased. Plasma creatinine and mean arterial pressure were elevated in the CRF groups when compared with control subjects (Table 1). The plasma immunoreactive endothelin-1 concentration was higher in CRF patients (3.9 ± 0.2 pg/ml, $N = 9$) than in control subjects (2.9 ± 0.2 pg/ml, $N = 10$, $P = 0.0001$). Similarly, the plasma immunoreactive big endothelin-1 concentration was higher in the CRF patients (43.5 ± 4.0 pg/ml, $N = 9$) than in control subjects (31.4 ± 3.5 pg/ml, $N = 10$, $P = 0.03$).

Infusion of norepinephrine and endothelin-1

Norepinephrine caused a dose-dependent reduction in FBF (Fig. 1 and Table 2) with a change in blood flow at the highest dose of −33 ± 7% in patients with CRF and −43 ± 7% in control subjects, which was similar between the two groups ($P = 0.53$). Saline infusion following the norepinephrine infusion allowed FBFs to return to baseline flows of 2.8 ± 0.3 ml/100 ml/min before endothelin-1 and 3.1 ± 0.6 ml/100 ml/min before norepinephrine ($P = 0.40$) for patients and, similarly, 2.9 ± 0.2 ml/100 ml/min before endothelin-1 and 3.4 ± 0.4 ml/100 ml/min before norepinephrine ($P = 0.24$) for control subjects. Endothelin-1 caused a progressive reduction in FBF (Fig. 2 and Table 2) with a change in blood flow after 60 minutes of infusion of −35 ± 6% in patients with CRF and −36 ± 5% in control subjects ($P = 0.81$).

Table 1. Subject characteristics

	Norepinephrine/ endothelin-1		BQ-123		Phosphoramidon		Thiorphan	
	Control	CRF	Control	CRF	Control	CRF	Control	CRF
Number	8	8	8	8	6	6	6	6
Age *years*	46 ± 5	47 ± 6	46 ± 5	47 ± 6	40 ± 8	41 ± 8	40 ± 8	41 ± 8
Sex *M/F*	7/1	7/1	7/1	7/1	6/0	6/0	6/0	6/0
Hemoglobin *g/dl*	12.7 ± 0.5	11.1 ± 0.8	12.7 ± 0.5	11.1 ± 0.8	13.0 ± 0.6	11.6 ± 0.7	13.0 ± 0.6	11.6 ± 0.7
Creatinine *μmol/liter*	87 ± 5	391 ± 54[a]	87 ± 5	391 ± 54[a]	84 ± 7	449 ± 66[a]	84 ± 7	449 ± 66[a]
Mean arterial pressure *mm Hg*	88 ± 3	104 ± 2[a]	85 ± 3	100 ± 4[a]	86 ± 4	110 ± 6[a]	87 ± 6	108 ± 4[b]

Results are expressed as mean ± SEM.
[a] $P < 0.002$, [b] $P \leq 0.05$ when compared to control subjects

Table 2. Forearm blood flows (FBF) before and during infusions

	Norepinephrine		Endothelin-1		BQ-123		Phosphoramidon		Thiorphan	
	Control	CRF	Control	CRF	Control	CRF	Control	CRF	Control	CRF
Control arm FBF *ml/100 ml/min*										
Basal	2.4 ± 0.2	3.0 ± 0.6	2.7 ± 0.3	2.8 ± 0.5	2.4 ± 0.3	3.2 ± 0.4	2.9 ± 0.4	3.4 ± 0.4	1.7 ± 0.2	3.9 ± 0.7[a]
End of infusion	2.9 ± 0.3	2.8 ± 0.6	3.2 ± 0.4[b]	3.0 ± 0.7	2.1 ± 0.3	3.5 ± 0.6[a]	3.6 ± 0.7	3.5 ± 0.4	2.5 ± 0.3[b]	2.8 ± 0.7[c]
Infused arm FBF *ml/100 ml/min*										
Basal	3.0 ± 0.3	3.1 ± 0.6	2.9 ± 0.2	2.8 ± 0.3	2.6 ± 0.3	3.7 ± 0.5	2.5 ± 0.4	4.6 ± 0.8[a]	1.9 ± 0.2	5.4 ± 1.3[a]
End of infusion	2.0 ± 0.2[b]	1.8 ± 0.2[b]	2.4 ± 0.3[b]	1.8 ± 0.3[b]	3.2 ± 0.4[b]	4.5 ± 0.7[b]	7.4 ± 0.8[b]	6.9 ± 0.7[b]	2.4 ± 0.3[b]	3.9 ± 0.7[b,c]

[a] $P < 0.05$ for resting blood flow when compared to control subjects (*t*-test)
[b] $P < 0.05$, compared to basal value (paired *t*-test)
[c] $P < 0.05$, compared to control response to infusion (ANOVA)

Infusion of BQ-123

Infusion of BQ-123 caused a progressive increase in FBF (Fig. 3 and Table 2), maximal at 60 minutes, of 11 ± 4% in patients with CRF and 44 ± 10% in control subjects; the response in patients with CRF was significantly reduced (Fig. 3) when compared with that of the control subjects ($P = 0.02$). In those subjects undergoing the two-hour infusion, there was no further vasodilation after 60 minutes of BQ-123 in either patients (13 ± 11% at 60 min, −3 ± 3% at 120 min, $P = 0.35$) or control subjects (46 ± 11% at 60 min, 48 ± 9% at 120 min, $P = 0.76$), respectively.

Infusion of phosphoramidon and thiorphan

Infusion of phosphoramidon caused a progressive increase in FBF (Fig. 4 and Table 2), maximal at 90 minutes, of 68 ± 20% in patients with CRF and 181 ± 41% in control subjects. The response of the patients with CRF to phosphoramidon was reduced (Fig. 4) when compared with that of the control subjects ($P = 0.001$). Infusion of thiorphan caused a progressive reduction in FBF (Table 2), maximal at 90 minutes, of −22 ± 6% in patients with CRF and −14 ± 6% in control subjects and was similar when comparing the two groups ($P = 0.39$; Fig. 4).

DISCUSSION

We have shown, using the ECE inhibitor, phosphoramidon, and the ET_A antagonist, BQ-123, that endogenous endothelin-1 contributes to the maintenance of resting forearm vascular tone in patients with CRF. We have also demonstrated, using BQ-123, that the contribution of endothelin-1 to resting vascular tone mediated through the ET_A receptor is decreased in patients with CRF compared with healthy controls. In addition, using phosphoramidon, the reduced contribution of endothelin-1 to the maintenance of vascular tone in patients with CRF appears to be due at least in part to reduced generation of endothelin-1. These functional responses are in contrast to the observed elevated concentrations of endothelin-1 and big endothelin-1 in CRF.

These studies involved infusion of locally but not systemically active doses of vasoactive agents into the forearm vascular bed. Indeed, FBF in the noninfused arm did not change during any infusion period apart from when endothelin-1 and thiorphan were infused in control subjects (Table 2), when the FBF in the control arm increased and the blood flow in the infused forearm decreased. The use of locally active doses of vasoactive agents is important in these studies, as systemic doses may obscure any direct vascular action through effects on other organs, such as the heart and kidney, or through

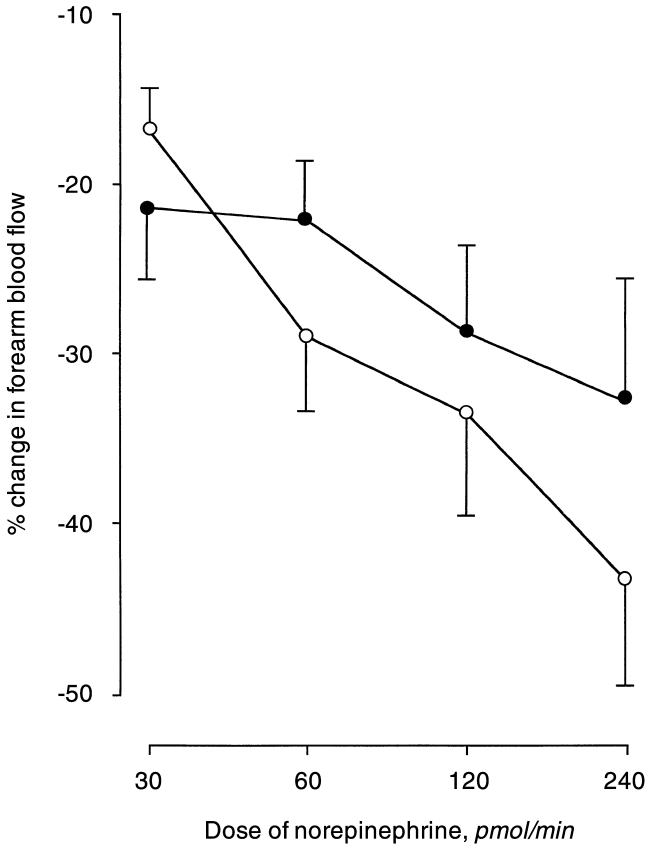

Fig. 1. Effect of norepinephrine infusion on forearm blood flow (FBF). Symbols are: (●) patients with CRF and (○) healthy control subjects ($P = 0.53$ by ANOVA).

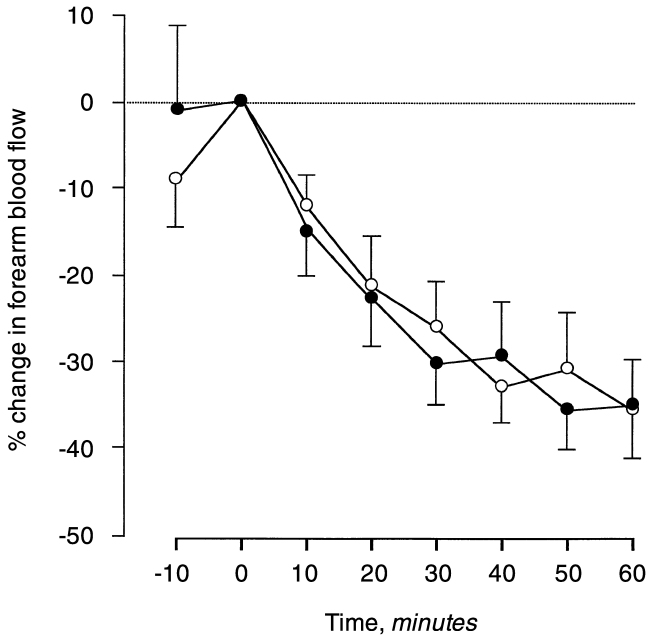

Fig. 2. Effect of endothelin-1 infusion on FBF. Symbols are: (●) patients with CRF and (○) healthy control subjects ($P = 0.81$ by ANOVA).

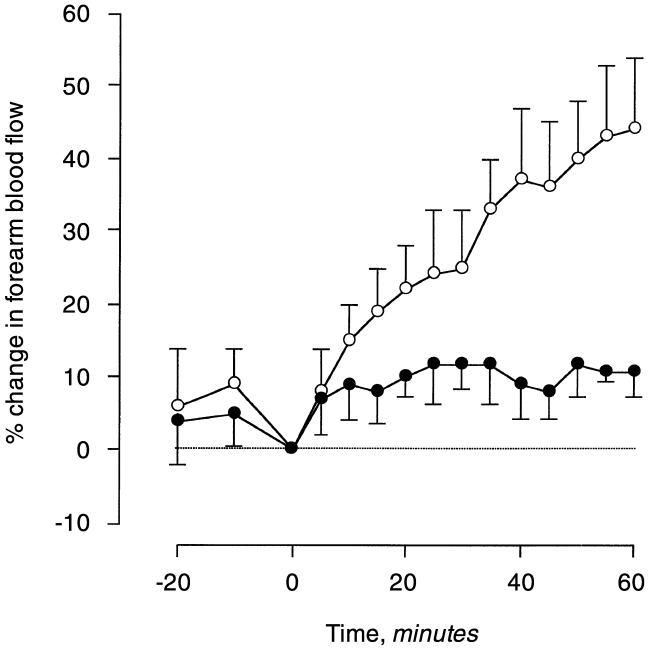

Fig. 3. Effect of BQ-123 infusion on FBF. Symbols are: (●) patients with CRF and (○) healthy control subjects ($P = 0.02$ by ANOVA).

activation of reflex mechanisms caused by changes in blood pressure [25]. The forearm vascular bed was chosen because the responses to vasoactive agents within this vascular bed are thought to be broadly representative of responses in other functionally important resistance beds [25, 26].

In contrast to the impaired responsiveness of the forearm resistance vessels to norepinephrine in patients with CRF who are dialysis dependent and anemic [21], there was no difference in the responsiveness to norepinephrine of the forearm resistance vessels between the dialysis-independent CRF patients and control subjects in this study. Also, in response to infusion of endothelin-1, there was a progressive vasoconstriction that was of a similar magnitude in the patients and control subjects, suggesting that responsiveness to endothelin-1 is not reduced in CRF in the way it is in chronic heart failure [27]. In addition, similar vascular responsiveness to infusion of norepinephrine and endothelin-1 in CRF patients and control subjects implies vascular functional integrity with respect to these two endogenous vasoactive mediators.

The specific ET_A receptor antagonist BQ-123 produced a progressive vasodilation in both patients with CRF and the control subjects, implying that endothelin-1, acting through the ET_A receptor, has a role in the maintenance of resting vascular tone. Furthermore, we have demonstrated, in both patients and controls, that the response to BQ-123 was maximal at 60 minutes. However, the response to BQ-123 in the patient group was significantly impaired when compared with control subjects. This im-

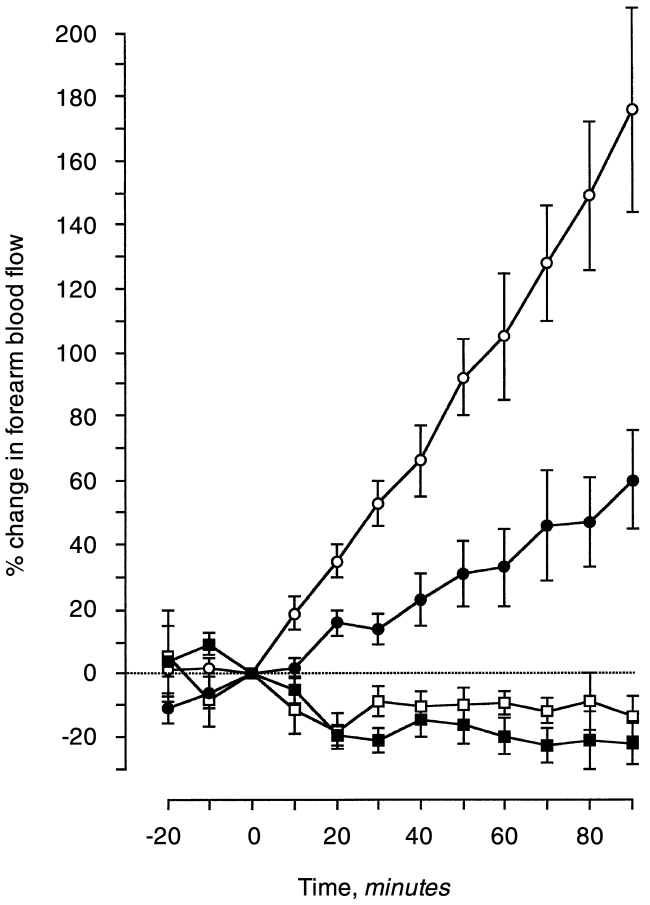

Fig. 4. Effect of phosphoramidon and thiorphan infusion on FBF. Symbols are: patients with CRF receiving phosphoramidon (●) and thiorphan (■) and healthy control subjects receiving phosphoramidon (○, $P = 0.001$ by ANOVA) and thiorphan (□, $P = 0.39$ by ANOVA).

paired response may have occurred for several reasons. First, elevated circulating concentrations of biologically active endothelin-1, either as a result of increased generation or decreased clearance, may have been sufficiently high that the dose of BQ-123 used was not sufficient to block the ET_A receptor. However, because the dose of BQ-123 infused was at least 10-fold higher than that previously shown to cause a maximal effect in the forearm in healthy subjects [13, 14], this is unlikely to be the explanation.

Second, in renal failure, endothelin-1 may cause vasoconstriction by acting on the ET_B receptor. ET_B agonists can cause vasoconstriction [6]. Our observations in renal failure may be consistent with those in chronic heart failure where there appears to be a relative up-regulation of the constrictor ET_B receptor [27, 28]. ET_B-mediated vasoconstriction in CRF may be of greater importance than in heart failure because ET_B-mediated vasodilation may be reduced, perhaps as a result of endothelial dysfunction, in CRF patients as compared with healthy subjects. However, our studies were not designed to fully investigate potential changes in ET_B receptor function in CRF.

Third, the altered response to infused BQ-123 in CRF may occur as a consequence of diminished responsiveness of the vascular smooth muscle cell in CRF. However, our findings of similar responsiveness to exogenous endothelin-1 between patients with CRF and control subjects are against this hypothesis.

Fourth, despite the increased concentrations of immunoreactive endothelin-1 and big endothelin-1 observed by our experiments and others [9], concentrations of biologically active endothelin-1 may be decreased in patients with CRF as a consequence of impaired generation of endothelin-1. Fractionation of plasma samples reveals that the elevation in immunoreactive endothelin-1 in CRF may be due to marked increases in immunoreactive big endothelin-1 degradation products [29] rather than biologically active endothelin-1. Furthermore, Shichiri reported similar concentrations of immunoreactive endothelin-1 in patients with renal failure and healthy control subjects when using an assay that does not cross-react with either big endothelin-1 or the C-terminal fragment [30].

Fifth, the increased blood pressure observed in the patients with CRF may contribute to the different responses between the CRF group and the control subjects rather than the altered renal function. However, this is unlikely because a study similar to ours found no difference in response to BQ-123 when comparing hypertensive subjects with normal renal function and control subjects [13]. Also against this is the inverse relationship between blood pressure and forearm vascular responsiveness to endothelin-1 in patients with dialysis-dependent renal failure using human recombinant erythropoietin [21].

Sixth, BQ-123–induced vasodilation may not only occur as a consequence of abolition of a constrictor stimulus but may, in part, be mediated by nitric oxide–dependent vasodilator tone [31]. The reduced response to BQ-123 observed in the CRF subjects may reflect an abnormality in nitric oxide-mediated vascular tone, possibly as a consequence of impaired endothelial function as described in patients with end-stage renal failure [32].

Infusion of the NEP and ECE inhibitor, phosphoramidon, caused forearm vasodilation. In contrast, infusion of the selective NEP inhibitor thiorphan caused a slow-onset vasoconstriction that was similar between the patients with CRF and control subjects. The vasodilator effect of the infused phosphoramidon was therefore likely to be an action of inhibiting ECE rather than NEP [4]. Vasodilation of the forearm vasculature in response to phosphoramidon suggests that endothelin-1 generation by the vascular endothelium is important in the maintenance of resting vascular tone in healthy subjects and shows that this is also the case in patients with CRF, although to a lesser extent. Given that constriction to

endothelin-1 was identical in CRF patients and controls, the implication is that there may be reduced generation of endothelin-1 in patients with CRF. However, this interpretation must be accepted with some caution because our renal failure patients had higher resting FBFs than control subjects, which may have reduced the concentration, and responses, to phosphoramidon. Also, the phosphoramidon responses observed in this study were greater than responses observed previously [4, 27]. In support of our conclusion, however, responses in patients to phosphoramidon and BQ-123 were reduced using the percentage change in FBF as a ratio of the infused to noninfused arm, this measure being less sensitive to differences in baseline blood flow [33]. In addition, the resting FBFs in the renal failure patients receiving BQ-123 and phosphoramidon were similar (infused arms, $P = 0.36$; noninfused arms, $P = 0.80$; Table 2), allowing direct comparison of inhibition of endothelin generation with phosphoramidon to blockade of the ET_A receptor with BQ-123. The greater increase in FBF in renal failure patients caused by phosphoramidon is consistent with an important vasoconstrictor role for the ET_B receptor in the maintenance of resting vascular tone in patients with CRF, although further studies with selective ET_B antagonists are required to confirm this observation.

In conclusion, we have confirmed elevated concentrations of immunoreactive endothelin-1 and big endothelin-1 in CRF. Furthermore, we have demonstrated that endogenous generation of endothelin-1 in the forearm plays an important role in the maintenance of resting vascular tone in healthy subjects and in patients with CRF who are independent of renal replacement therapy. Also, we have demonstrated that the contribution of endothelin-1 to resting vascular tone is decreased in CRF as a consequence of reduced generation of endothelin-1. However, our findings also suggest that the use of ECE inhibitors may be of greater potential for control of hypertension associated with CRF than selective ET_A receptor antagonists. Further studies with systemic and chronic dosing are needed to explore these issues.

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