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Chronic Renal Failure Leads to Reduced Flow-Dependent Dilation in Isolated Rat Skeletal Muscle Arterioles due to Lack of NO Mediation

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Key Words
Chronic renal failure · Arteriole · Flow-dependent dilation · Nitric oxide · Partial nephrectomy

Abstract
Background: Chronic renal failure (CRF) is frequently accompanied by systemic vascular alterations which further increase the morbidity and mortality of these patients. However, the nature and the underlying mechanisms of vascular dysfunction are not completely understood. We hypothesized that – in addition to other factors – CRF alters local vasomotor mechanisms that are intrinsic to the vascular wall. ***Methods:*** Changes in the diameter of isolated, pressurized (at 80 mm Hg) gracilis skeletal muscle arterioles (diameter approximately 150 μm) of female Wistar rats were investigated by videomicroscopy. Arteriolar responses to an increase in flow and vasoactive agents in partially nephrectomized (NX) and sham-operated (control) rats were compared. ***Results:*** In NX rats, serum creatinine and urine protein excretion were increased. Compared to controls, increases in intraluminal flow (from 0 to 40 μl/min) resulted in significantly reduced dilation in arterioles of NX rats (maximum: 32 ± 4 vs. 15 ± 4 μm, $p < 0.05$). Inhibition of nitric oxide (NO) synthesis with L-NAME reduced the dilation of control arterioles but did not affect responses of NX arterioles. Also, dilations in response to histamine were significantly reduced in arterioles from NX rats as compared to control rats. L-NAME significantly decreased histamine-induced dilations of control arterioles, but it did not affect responses of NX arterioles. Dilations in response to the NO donor sodium nitroprusside were also significantly decreased in NX arterioles as compared to responses of control vessels, whereas responses to adenosine and norepinephrine were not significantly different in the two groups. ***Conclusions:*** We conclude that in rat skeletal muscle arterioles, CRF induced by renal mass reduction alters the mechanosensitive and agonist-induced responses of peripheral arterioles, in part by interfering with NO-signaling mechanisms. These alterations could contribute to increased peripheral vascular resistance and further aggravate the cardiovascular complications in CRF.

Introduction

Clinical and experimental evidence shows that patients with chronic renal failure (CRF) have a significantly greater incidence of systemic vascular disease [1–3].

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Several mechanisms have been proposed by which CRF may alter the function and structure of peripheral vessels that could lead to an 'accelerated' systemic atherosclerosis and/or hypertension. The characteristics of vascular dysfunction and underlying mechanisms in CRF, however, are not fully elucidated [4, 5]. It has been proposed that CRF has multifactorial effects on various regulatory mechanisms governing the diameter of resistance vessels, and in addition to neural and humoral factors, local mechanisms intrinsic to the vascular wall [6] seem to be also affected [7–11].

Recently, in patients with CRF, Morris et al. [8, 9] have shown that increases in forearm blood flow in response to carbachol – but not to the nitric oxide (NO) donor sodium nitroprusside (SNP) – were attenuated, and in arterioles obtained from subcutaneous fat biopsies, acetylcholine (ACh)-induced NO-mediated dilations were decreased compared to responses of control vessels [9]. Moreover, Savage et al. [10, 11] have demonstrated that subtotal nephrectomy (an animal model of CRF) reduced the basal diameter of rat femoral artery and that an increase in intraluminal flow resulted in dilation of norepinephrine-preconstricted femoral arteries but constriction of control vessels. Other previous studies, however, showed dilation of large vessels in response to flow [12, 13]. More importantly, fewer data are available regarding the CRF-induced alterations of the vasoactive function of arterioles, known to be importantly involved in the regulation of vascular resistance, especially regarding the nature of the flow (shear stress)-dependent mechanism. This mechanism contributes to the moment-to-moment regulation of arteriolar tone [6, 14, 15] and development of peripheral vascular resistance.

On the basis of previous findings, we hypothesized that CRF alters the flow/shear stress-dependent dilation of arterioles by impairing the signaling pathway involving NO. To test this hypothesis, flow-induced dilation of gracilis muscle arterioles isolated from control (sham-operated) and partially nephrectomized (NX) female rats was investigated. To better evaluate possible changes in the NO signaling pathway in CRF, female rats were utilized, because previously we [16] and others [17, 18] have shown that compared to males, the contribution of NO to mediation of arteriolar responses is greater in female rats. In addition, the function of arteriolar endothelium and smooth muscle was assessed by vasoactive substances of known action.

Methods

CRF Model

Seven-week-old female Wistar (WU, RT1[l], Charles River Co.) rats weighing 160–210 g were used. All animals were kept under standard conditions and received water ad libitum. All procedures were in accordance with guidelines set by Institutional Animal Care and Use Committees. Rats were divided into two groups (n = 10 per group); animals in the first group underwent partial NX, while rats in the second group underwent sham operation and served as controls. The partial NX procedure has been described previously [19]. In brief, under diethyl ether anesthesia, the right kidney was decapsulated and removed through a midline laparotomy. In the same operative session, one third of the decapsulated left kidney was resected with scissors, leaving the pelvis and the hilum intact [20]. The excised kidney tissue was weighed on an analytic scale. The average reduction of total kidney mass was 70 ± 4.5%. Blood was collected from the aortas of rats fasted overnight. It was immediately cooled on ice and centrifuged at 3,000 *g* for 20 min at 4 °C. Serum and urine creatinine concentrations were determined using a Reflotron IV automate (Boehringer Mannheim, Germany). Creatinine clearance was calculated. Urinary protein concentrations were determined with the Biuret method using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, Munich, Germany). Absorbance was determined with a Philips PU8700 spectrophotometer at a wavelength of 595 nm. Twenty-four weeks after partial NX and overnight fasting, arterial blood pressures were measured in anesthetized rats via a cannula inserted into the femoral artery.

Isolation of Arterioles

Experiments were conducted on isolated arterioles (inside diameter approximately 150 μm) of rat gracilis muscle as described previously [21]. Briefly, gracilis muscle of diethyl ether-anesthetized rats was exposed and isolated from surrounding tissues with microsurgical instruments and an operating microscope. A segment approximately 1.5 mm in length of an arteriole running intramuscularly was isolated and transferred into an organ chamber containing two glass micropipettes filled with physiological salt solution. Arterioles were then cannulated on both sides in an organ chamber and were continuously superfused with physiological salt solution (in mmol/l: 110 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.0 $MgSO_4$, 1.0 KH_2PO_4, 10.0 dextrose and 24.0 $NaHCO_3$; equilibrated with 10% O_2, 5% CO_2 and 85% N_2, at pH 7.4). Inflow and outflow pressures were measured by an electromanometer. The temperature was set at 37 °C by a temperature controller and the vessel was allowed to develop spontaneous tone in response to intraluminal pressure (80 mm Hg) under no-flow conditions (equilibration period 1 h). The inside diameter of arterioles was measured by videomicroscopy [21] and recorded on a chart recorder (Cole-Parmer, Vernon Hills, Ill., USA).

Flow- and Agonist-Induced Responses

Arteriolar diameters were measured in response to step increases in intraluminal flow (from 0 to 40 μl/min). Intraluminal flow was established at a constant intravascular pressure (80 mm Hg) by changing the inflow and outflow pressure to an equal degree, but in opposite directions, to keep midpoint luminal pressure constant, and the flow was measured with a ball flowmeter (Omega Engineering Inc., Stamford, Conn., USA). Each flow rate was maintained for 5 min to allow the vessel to reach a steady-state diameter. In separate experiments, peak arteriolar responses to cumulative doses of hista-

Table 1. Effects of partial NX on body weight, renal function and arteriolar diameters

	Control (24 weeks)	NX (4 weeks)	NX (24 weeks)
Body weight, g	297±7	234±2	292±6
Serum creatinine, μmol/l	<44	51±5	59±4*
Creatinine clearance, ml/min	–[1]	0.6±0.3	0.8±0.6
Urine protein, mg/24 h	33±7	41±10	283±47*
Active arteriolar diameter, μm	166±4	n.a.	136±9*
Passive arteriolar diameter, μm	206±3	n.a.	188±15

Values are mean ± SEM. Values were obtained in sham-operated rats (control, n = 10) and in rats with partial NX (n = 10) after 4 and 24 weeks. n.a. = Not available. * $p < 0.05$ versus control.

[1] Normal serum creatinine was under the detection level (<44 ml/min) of the assay used (Reflotron®).

mine (3×10^{-6}–10^{-4} mol/l), SNP (10^{-9}–10^{-6} mol/l), adenosine (10^{-6}–10^{-4} mol/l) and norepinephrine (3×10^{-9}–3×10^{-7} mol/l) were measured. In both flow and agonist experiments, after obtaining control responses, the vessels were then incubated with N^{ω}-nitro-*L*-arginine-methyl-ester (L-NAME; 10^{-4} mol/l for 30 min), an inhibitor of NO synthase (NOS), and flow- and agonist-induced responses were reassessed. All drugs were added to the vessel chamber and final concentrations are reported. All salts and chemicals were obtained from Sigma-Aldrich Co., and solutions were prepared on the day of the experiment.

Data Analysis

Flow-induced responses are expressed as changes in arteriolar diameter (in absolute values), and agonist-induced dilations are expressed as a percentage of the maximum dilation of the vessel, defined as the passive diameter at 80 mm Hg intraluminal pressure in a Ca^{2+}-free medium containing 10^{-3} mol/l EGTA and 10^{-4} mol/l SNP. Wall shear stress (WSS) was calculated by the formula $4\eta Q/r3$, where η is the viscosity of the perfusate (0.007 poise at 37 °C), Q is the perfusate flow, and r is the vessel radius. Data are expressed as means ± SEM. Statistical analyses were performed by two-way analysis of variance for repeated measures followed by the Tukey post hoc test or Student's t test, as appropriate. Statistical significance was considered present at $p < 0.05$.

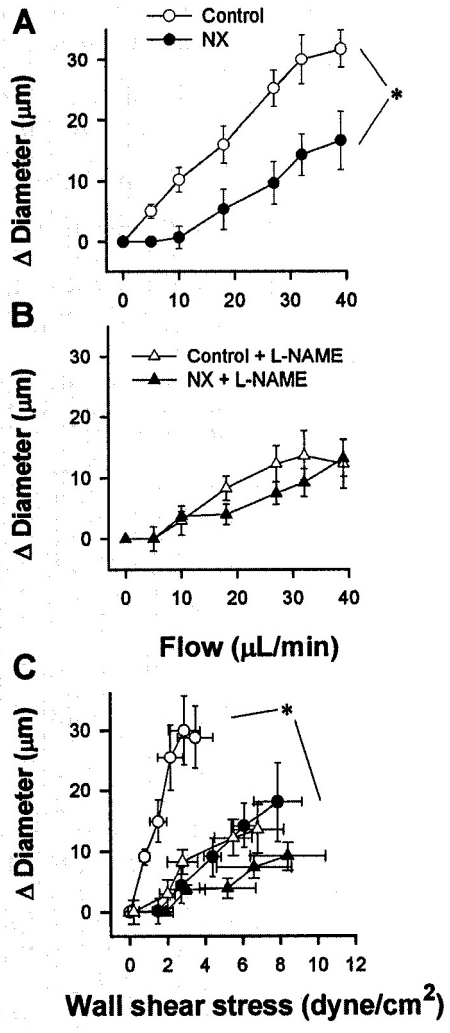

Fig. 1. A, B Changes in diameter of skeletal muscle arterioles of control (sham-operated) and partially NX rats as a function of intraluminal flow in the absence (**A**) and presence (**B**) of the eNOS inhibitor L-NAME. Data are mean ± SEM (n = 10). **C** Changes in arteriolar diameter as a function of calculated wall shear stress in the absence or presence of L-NAME in control and NX rats. * $p < 0.05$.

Results

Functional Parameters

Body weights of partially NX and control animals were similar at week 24 after NX (table 1). By week 24, proteinuria had developed in NX rats. Compared to week 4, at week 24, serum creatinine was significantly higher, but there were no significant changes in creatinine clearance. Sham-operated control rats exhibited normal values (table 1). As compared to controls, arterial blood pressure, measured during anesthesia, was significantly higher in NX rats (78 ± 6 vs. 96 ± 4 mm Hg; table 1), confirming previous findings obtained under the same conditions [10, 11]. Arterioles isolated from gracilis muscle of control and NX rats developed an active tone in response to

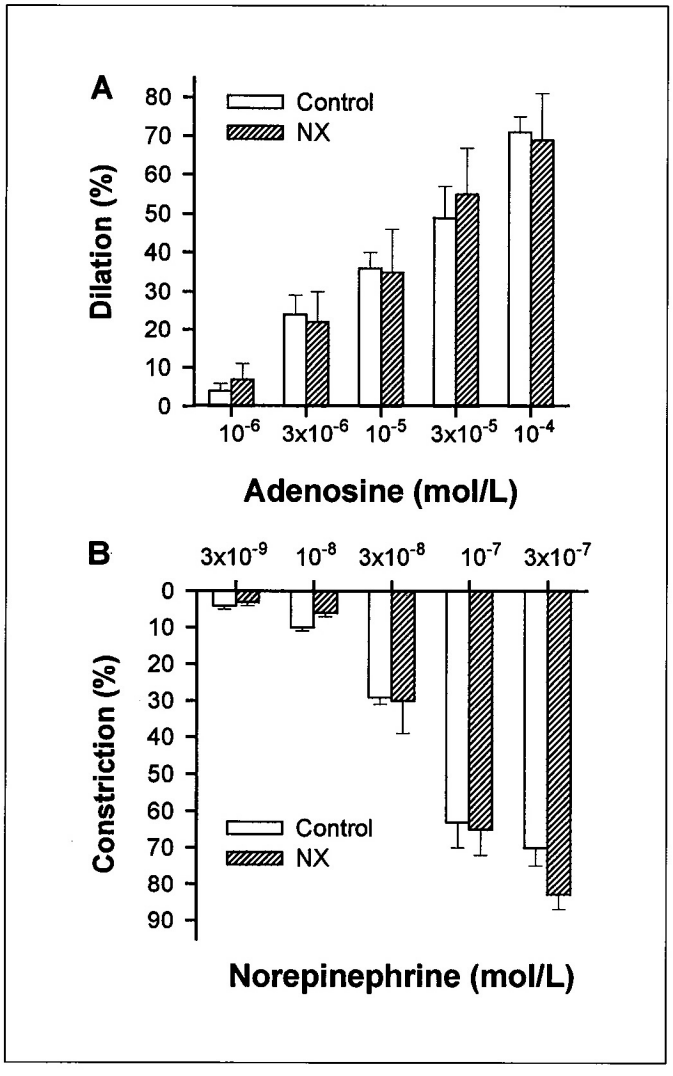

Fig. 2. Dilations in response to cumulative doses of adenosine (**A**) and constrictions in response to cumulative doses of norepinephrine (**B**) in skeletal muscle arterioles of control (sham-operated) and partially NX rats. Data are mean ± SEM (n = 10).

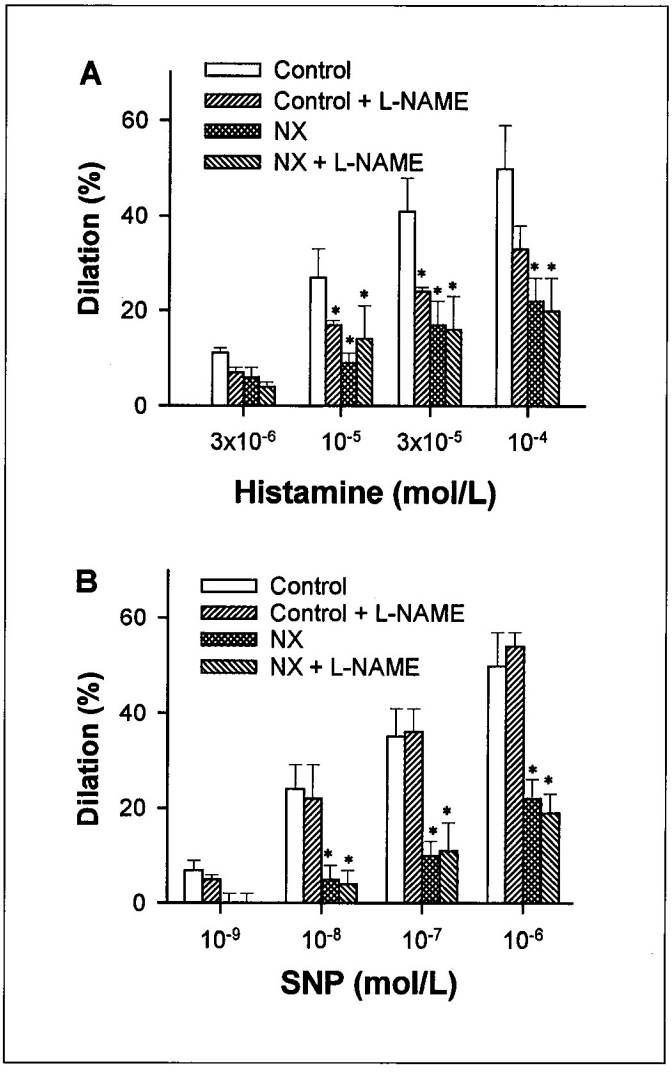

Fig. 3. Dilations in response to cumulative doses of histamine (**A**) and SNP (**B**) in skeletal muscle arterioles of control (sham-operated) and partially NX rats in the absence or presence of the eNOS inhibitor L-NAME. Data are mean ± SEM (n = 10). * $p < 0.05$.

80 mm Hg intraluminal pressure without the use of any vasoactive agent. The basal diameter (at 80 mm Hg) of arterioles isolated from NX rats was significantly lower than that of controls, whereas there was no significant difference between the passive diameters (see Methods) of control and NX animals (table 1).

Flow-Induced Arteriolar Responses

Increases in flow elicited substantial dilations in control arterioles that were significantly reduced in NX vessels (maximum 32 ± 4 vs. 15 ± 4 μm, respectively; fig. 1A). Inhibition of endothelial NOS (eNOS) with L-NAME significantly reduced (by approximately 60%) the flow-induced dilation in control arterioles, but did not affect the reduced flow-induced dilation of NX arterioles (fig. 1B). The remaining dilations of arterioles after inhibition of eNOS are mediated by dilator prostaglandins, as shown by our previous studies [21]. Changes in diameter of arterioles of control and NX rats as a function of wall shear stress demonstrate that in control arterioles, the

substantial increases in diameter prevent great increases in wall shear stress. In contrast, the reduced increases in the diameter of untreated NX and L-NAME-treated control and NX arterioles resulted in higher wall shear stress compared to that of untreated control vessels (fig. 1C).

Arterial Responses to Vasoactive Agents

To provide further evidence for the alteration in NO mediation of arteriolar responses, in a separate group of experiments, the function of endothelium and smooth muscle cells was assessed by responses to vasoactive agents of known actions. Adenosine elicited similar dilations in control and NX arterioles in a dose-dependent manner (10^{-6}–10^{-4} mol/l) (fig. 2A). Similarly, constrictions in response to norepinephrine (3×10^{-9}–3×10^{-7} mol/l) were not significantly different between arterioles of control and NX rats (fig. 2B). However, histamine (3×10^{-6}–10^{-4} mol/l) and NO donor SNP (10^{-9}–10^{-6} mol/l) in a dose-dependent manner elicited significantly reduced dilation of NX arterioles as compared to controls (fig. 3A, B). After inhibition of eNOS with L-NAME, histamine-induced dilations became significantly reduced in control arterioles, but they remained unaffected in NX vessels (fig. 3A). L-NAME did not affect dilations in response to SNP either of control or NX arterioles (fig. 3B).

Discussion

The salient findings of this study are that in skeletal muscle arterioles isolated from rats with CRF (elicited by partial NX), flow/shear stress-, histamine- and the NO donor SNP-induced dilations are significantly reduced, likely due to impaired NO mediation, whereas responses to adenosine and norepinephrine remain unaltered.

CRF frequently leads to peripheral vascular diseases such as 'accelerated' atherosclerosis and/or hypertension [1–3], but the nature of the underlying pathological mechanisms is not completely understood [22]. It is likely that first functional changes occur, which are, however, not well defined in arterioles in CRF [7–11]. Elucidating the causes of vascular dysfunction in CRF is difficult, due to the presence of several other confounding factors, such as accompanying diseases and dialysis treatment. Thus, in the present study, we aimed to elucidate the vasomotor function of arterioles at an early stage of CRF induced by partial NX, an animal model used to study CRF [10, 11, 20]. In this model, the effects of CRF can be investigated without the confounding effects of other diseases and substantial vascular morphological changes. After partial NX, by week 24, proteinuria developed, together with increased serum creatinine (table 1), whereas creatinine clearance and arterial blood pressure measured during anaesthesia remained within the normal range (table 1), indicating an early stage of renal failure. Indeed, it has been shown that in partially NX animals, as utilized in the present study, the hyperfiltration injury primarily destroys glomerular structure, resulting in protein leakage into the urine, although it may not reduce glomerular filtration, hence creatinine clearance remains in the normal range [23].

Most previous studies describing alterations in vasodilator function in CRF were conducted in large vessels and relied on the characterization of vascular responses only to pharmacological agents [9–11]. Several vasoactive substances (ACh, norepinephrine, adenosine, etc.) with known mechanisms of action have been used to test endothelium-dependent or- independent functions. Dilations in response to ACh were reduced compared to control in isolated norepinephrine-preconstricted vessels obtained from fat tissue biopsies of patients with CRF, and an impaired NO mediation of responses was suggested [8]. In contrast, in preconstricted mesenteric arteries isolated from rats with subtotal NX, ACh-induced dilations were not significantly affected [24]. Similarly, Thuraisingham and Raine [25] found that arteriolar dilation in response to ACh was unaltered in uremic rats with subtotal NX, suggesting a maintained NO mediation of mesenteric arteries in CRF.

Less is known regarding the functional changes of skeletal muscle arterioles, which represent a major portion of peripheral resistance. Thus, we utilized gracilis muscle arterioles isolated from partially NX and control female rats and characterized the flow- and agonist-induced responses. Arterioles have the unique ability to substantially change their diameter in response to changes in hemodynamic forces, which frequently occur in vivo [15], and thus they are suitable to study functional changes of peripheral vessels. It has been shown that increases in intraluminal flow increase wall shear stress, resulting in dilation of arterioles, which in turn reduces shear stress in a negative feedback manner, providing for a functional adaptation to high-flow conditions [6, 15]. The physiological role of this mechanism is to provide for a coordinated decrease in upstream resistance in the microvascular network, resulting in a substantial increase in blood flow to supply adequate blood to parenchymal tissue in case of increased demand (e.g. exercise, collateral flow development, etc.). It is likely that shear stress-sensitive mecha-

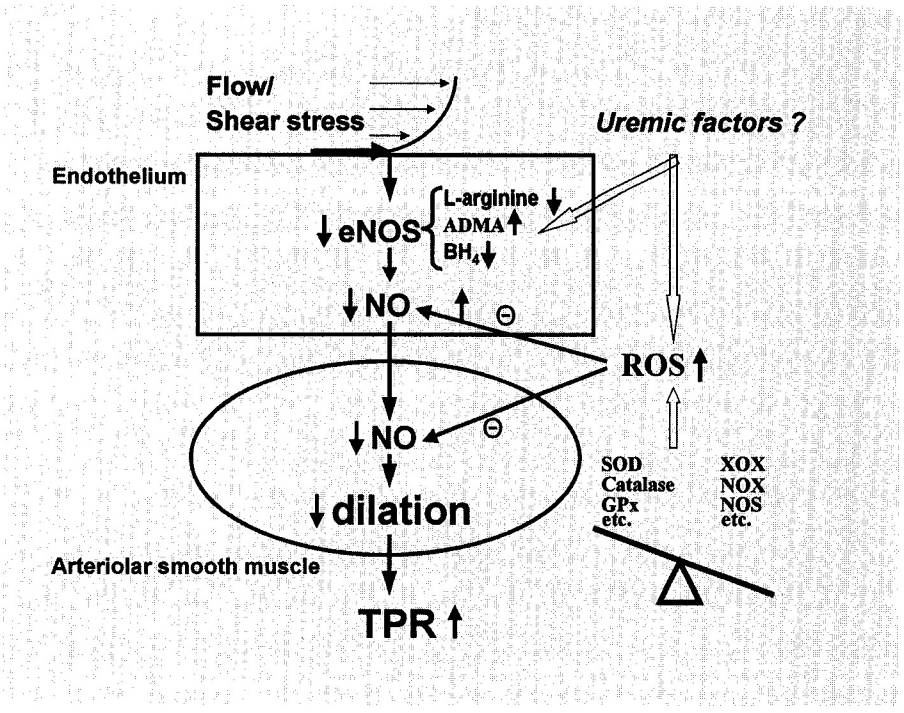

Fig. 4. Proposed scheme by which CRF alters endothelial functions resulting in reduced NO mediation of flow/shear stress-induced dilation in isolated skeletal muscle arterioles. ADMA = Asymmetric dimethylarginine; BH_4 = tetrahydrobiopterin; ROS = reactive oxygen species; SOD = superoxide dismutase; GPx = glutathione peroxidase; XOX = xanthine oxidase; NOX = NAD(P)H oxidase; TPR = total peripheral resistance.

nisms can participate in the pathogenesis of various disease states (such as arteriosclerosis, diabetes mellitus and hypertension [6]) or be changed as a consequence of them.

However, there are only a limited number of studies investigating flow/shear stress-dependent responses in CRF and the findings are not completely understood [11]. The present study conducted in isolated skeletal muscle arterioles showed that stepwise increases in intraluminal flow elicited substantial dilations, confirming previous findings [21]. In these arterioles, flow-dependent dilation is mediated by the corelease of NO and dilator prostaglandins [21]. We have also found that arterioles of NX rats exhibited enhanced basal tone (developed to 80 mm Hg) compared to that of control rats (table 1), which accords with earlier findings [11]. An important new finding of the present study is that flow-dependent dilation was significantly reduced in arterioles of NX rats as compared to vessels of control, sham-operated rats (fig. 1A). Extrapolating this in vitro finding to in vivo conditions, one can speculate that resistance vessels of individuals with renal failure are exposed to higher wall shear stress than those of normals, resulting in a greater power dissipation in their cardiovascular system. If so, this could leave constrictor mechanisms partially unopposed, which could ultimately lead to a higher systemic blood pressure in the later phase of CRF.

Because inhibition of eNOS by L-NAME did not further affect flow-induced dilations of NX arterioles, it is likely that the reduced flow-dependent dilation is due to the lack of NO mediation of the response (fig. 1B). These findings, together with earlier observations [8], suggest that in CRF, there is an altered endothelial mechanosensitive shear stress function due to the impairment of NO mediation.

To substantiate further that NO mediation of arteriolar responses is altered in CRF, in a separate group of experiments, the function of endothelium and smooth muscle cells was assessed by responses to vasoactive agents of known actions. We found that arteriolar constrictions in response to norepinephrine and dilations in response to adenosine were not significantly different between control and NX rats (fig. 2A, B), suggesting that the vasomotor function of arteriolar smooth muscle is not impaired in general [7, 8, 24]. However, histamine elicited significantly reduced dilations in NX arterioles compared to those of normal arterioles (fig. 2C). Inhibition of eNOS reduced the histamine-induced dilations in control arterioles, but not in NX vessels. After inhibition of eNOS, dilations in response to histamine became equal to

those observed in NX vessels, suggesting that the reduced dilation in response to histamine in NX arterioles was due to decreased mediation of responses by NO. To gain further insight into the nature of the mechanisms responsible for the impaired mediation of responses by NO, responses to the NO donor SNP were investigated. Interestingly, we found that in NX arterioles, dilations induced by the NO donor SNP were also reduced compared to those of controls. Inhibition of eNOS by L-NAME did not affect SNP-induced responses of either control or NX arterioles.

Several mechanisms could be responsible for the impaired NO mediation in CRF. On the basis of present and previous findings, we developed a model describing the possible pathomechanisms which could contribute to the development of dysfunction of peripheral microvessels in CRF (fig. 4). Previous studies suggest an accumulation of endogenous inhibitors of eNOS, such as asymmetric dimethylarginine [26]. Although these substances are short-lived in the circulation, they may accumulate in endothelial cells [27]. Previous studies also proposed that in CRF, there are decreased levels of substrates and cofactors of eNOS, such as *L*-arginine and tetrahydrobiopterin, which could result in decreased NO synthesis [26]. Another possible mechanism by which NO mediation is reduced is the decrease in the bioavailability of NO due to an enhanced production of reactive oxygen species. This latter idea is supported by our findings that not only flow- and histamine-induced dilations, but also dilations in response to the NO donor SNP were reduced in CRF. Although several previous studies found unaltered dilations in response to SNP or glycerine trinitrate in vessels from CRF [8, 24, 25], recently, Cupisti et al. [28] reported that in skin microvessels, the hyperemic response to SNP is decreased in patients with CRF. It has been shown that NO can rapidly react to reactive oxygen species and forms peroxynitrite [29]. Indeed, Vaziri et al. [30] showed a marked increase in the plasma lipid peroxidation product malondialdehyde, and this was accompanied by widespread accumulation of nitrotyrosine in various vascular tissues in CRF. However, further studies are needed to elucidate the exact mechanisms leading to reduced NO mediation of flow/shear stress-induced dilation and its role in the development of vascular dysfunction in CRF.

In conclusion, our findings demonstrate for the first time that in skeletal muscle arterioles of rats, CRF – due to impaired signaling by NO – results in dysfunction of endothelial regulation of wall shear stress, a pathomechanism that could contribute to the peripheral vascular diseases observed in CRF.

Acknowledgments

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