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The myogenic response in uremic hypertension

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Background. The constriction of resistance arteries in response to an increase in transmural pressure, the myogenic response, is thought to be an important determinant of peripheral vascular resistance and therefore of arterial blood pressure. Since raised peripheral resistance is known to occur in uremic hypertension, abnormal myogenic constriction might be responsible. We sought to assess the myogenic response of resistance arteries from the subtotal nephrectomy rat model of uremic hypertension.

Methods. Uremic Wistar-Kyoto (WKYU) rats, and sham-operated normotensive (WKYC) and spontaneously hypertensive (SHRC) controls were studied in parallel. Skeletal muscle arteries were mounted on a pressure myograph and allowed to develop myogenic constriction. The active internal diameter was measured at increasing lumen pressures from 20 to 200 mm Hg. Vascular smooth muscle then was relaxed in a calcium free solution containing nitroprusside, and the passive internal diameter measured at the same pressure steps. The ratio of active to passive diameter at any given pressure was used to assess the myogenic response.

Results. Myogenic constriction was not increased in either WKYU or SHRC compared to WKYC at pressures up to 180 mm Hg.

Conclusions. Increased myogenic tone is not the cause of uremic hypertension.

Hypertension usually accompanies chronic renal disease [1]. It accelerates the rate of progression of kidney failure [2] and contributes to the high incidence of stroke [3], heart failure [4], and overall cardiovascular mortality [5] seen in uremia.

There are several mechanisms by which chronic renal insufficiency might cause hypertension. These include sodium retention [6, 7], inappropriate activity of the renin-angiotensin-aldosterone system [8, 9], and increased sympathetic nervous system activity [10, 11]. Vascular endothelial dysfunction [12] and structural changes of resistance arteries [13, 14] also might increase blood pressure in uremia.

Key words: myogenic response, uremia, hypertension, myograph, resistance artery, remnant kidney.

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In common with other forms of hypertension, uremic hypertension is associated with a raised peripheral vascular resistance [15]. Most of the cardiovascular resistance to blood flow occurs in arteries of less than 200 to 300 μm diameter [16]. These so-called resistance vessels possess intrinsic contractility that is pressure-dependent; a rise in lumen pressure causes constriction and a fall in pressure causes dilation. This response was first described a century ago [17] and has been termed the "myogenic response." Steady-state myogenic tone probably accounts for a substantial proportion of the peripheral resistance [18] and, therefore, could be important in the maintenance of arterial pressure.

We hypothesized that increased myogenic tone might contribute to uremic hypertension. We studied the myogenic response of isolated pressurized skeletal muscle (cremaster) resistance arteries from uremic hypertensive Wistar-Kyoto rats (WKYU). Controls comprised vessels from both normotensive (WKYC) and spontaneously hypertensive (SHRC) animals, in an attempt to single out the effects of uremia.

METHODS

Animals

All procedures had the prior approval of the Home Office (Project License number 70/5014), and were performed in accordance with the Animals Scientific Procedures Act, 1986. Twelve- to 14-week-old male WKY and SHR were obtained from Harlan Ltd. (Shaw's Farm, Oxon, UK). General anesthesia was induced for both stages of nephrectomy by intramuscular injection of HYPNORM™ 1 mL/kg body weight (fentanyl citrate 0.315 mg/mL plus fluanisone 10 mg/mL; Janssen-Cilag Ltd., Bucks, UK), followed by intraperitoneal injection of diazepam 2.5 mg/kg (CP Pharmaceuticals Ltd., Wrexham, Wales, UK). Postoperative analgesia was provided by subcutaneous injection of buprenorphine 50 μg/kg (Schering-Plough Ltd., Middlesex, UK). Left subtotal nephrectomy through a midline abdominal incision removed approximately ¾ of the renal mass, with total right nephrectomy through a flank incision one week later. WKYC and SHR animals underwent sham surgery

in which the appropriate kidney was stripped of its capsule only. Animals were maintained for approximately three months during which WKYC and SHRC were pair fed with their WKYU partner; ethical considerations ensured that a minimum 20 g/day of standard rat chow was offered to controls. Several days before sacrifice, under halothane anesthesia (Concord Pharmaceuticals Ltd., Essex, UK), an abdominal aortic catheter (0.61 mm outer diameter and 0.28 mm inner diameter; Sims Portex Ltd., Kent, UK) was inserted retrogradely through the femoral artery, heparinized and exteriorized through the skin at the nape of the neck [19]. Postoperative analgesia was provided by subcutaneous injection of buprenorphine as required. Two to three days later conscious blood pressure was measured (pressure transducer model #60-3003; Harvard Apparatus, Boston, MA, USA). Blood was drawn for analysis, and the animal killed by cervical dislocation. The cremaster muscle was immediately excised at its most proximal aspect and placed in ice-cold physiological saline solution (PSS). One vessel was studied from each rat on the day of sacrifice. The muscle was pinned out on a silastic base within a petri dish containing cold PSS. The main branch (1A branch [19]) of the artery was identified and dissected from adjacent connective tissue.

Biochemical and hematological analysis

Plasma creatinine concentration was determined by the Jaffé rate method using a creatinine analyzer (Beckman Coulter Ltd., UK). Blood hemoglobin was measured using a β-hemoglobin photometer (HemoCue Ltd., Derbyshire, UK).

Drugs and solutions

All chemicals (Sigma-Aldrich Company Ltd., Dorset, UK) were made up on the day of the experiment in sterile distilled AnalaR water (Merck Ltd., Poole, Dorset, UK). PSS had the following composition (mmol/L): 119 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.17 KH_2PO_4, 1.17 $MgSO_4$, 2.0 $CaCl_2$ and 5.5 glucose. $CaCl_2$ was added to PSS immediately prior to use. Calcium free PSS contained ethyleneglycol–bis (β-aminoethylether)-N, N′-tetraacetic acid (EGTA) 1 mmol/L and sodium nitroprusside 100 μmol/L.

The pressure myograph

The method for assessing the myogenic response with the pressure myograph has been described [20]. This technique was chosen because it allows isolated vessels of precise anatomical location, devoid of circulating vasoactive factors, to be studied. Although endothelium and nerve endings remained intact within vessels, they have been shown to exert little or no effect on the myogenic response [21, 22]. The artery was mounted between the two glass microcannulas (tip diameter 100 μm) of the myograph (Living Systems Instrumentation, Burlington, VT, USA). First, the proximal end of the vessel was secured around the proximal cannula with a knotted nylon strand. A servo-controlled pump generated pressure within the vessel by delivering PSS to the proximal cannula. The pump continued to pressurize or de-pressurize the system until the pressure, sensed by a semiconductor transducer, equaled a predetermined level set by the user. Thus, the proximal cannula was first pressurized to approximately 10 mm Hg to flush blood from the vessel lumen, before being lowered to 5 mm Hg, while the distal end of the vessel was secured to the distal cannula as above. The vessel lumen was then slowly pressurized to 100 mm Hg, and tested for leaks. In a leak free system it is possible to switch off the pump with no dissipation of the previously generated pressure. If the vessel leaked, it was discarded. A reservoir of PSS was gassed with 95% O_2/5% CO_2, heated, and circulated at a rate of 40 mL/min to the vessel chamber, which was continuously monitored and maintained at pH 7.40 and 33°C, the temperature of the cremaster muscle in vivo. After being placed on the stage of an inverted microscope (Nikon TMS-F; Nikon Corporation, Tokyo, Japan), the vessel was viewed through a ×10 objective lens linked through a camera port to a video monitor. The distal cannula could be moved by a Vernier screw mechanism to allow precise control of the axial length of the mounted vessel. By this means the distance between cannula could be adjusted to remove kinks arising from pressure-induced changes in vessel length. The internal diameter of the vessel was measured with a video dimension analyzer.

Experimental protocol

Vessels developed myogenic tone during equilibration at a lumen pressure of 100 mm Hg. The pressure was then decreased to 20 mm Hg and at approximately 10-minute intervals the stable active internal diameter was recorded and the pressure increased by 20 mm Hg, up to a maximum of 200 mm Hg. Lumen pressure was then decreased to 100 mm Hg, and vascular myogenic tone abolished by superfusing the calcium-free PSS for 30 minutes. The pressure steps were then repeated, and the passive internal diameter measured at each increment.

Calculations

The myogenic response was represented as the active normalized diameter, which is the active internal diameter at a given pressure (D_a, myogenic constriction present) divided by the passive internal diameter at the same pressure (D_p, myogenic constriction abolished). By this means, a normalized diameter of 0.7 corresponds to a myogenic constriction of $100 \times (1.0 - 0.7) = 30\%$, relative to the passive diameter.

Statistical analysis

Values are expressed as the mean and by standard error in brackets. In all comparisons there were three

Table 1. Experimental animal data for WKYU ($N = 10$), WKYC ($N = 13$), and SHRC ($N = 13$)

	WKYU	WKYC	SHRC
Mean arterial pressure *mm Hg*	161 (8)[a]	139 (4)	193 (3)[c]
Blood hemoglobin *g/dL*	8.2 (0.6)[bc]	14.1 (0.5)	14.9 (0.5)
Final body weight *g*	298 (9)[bc]	361 (6)	366 (6)
Plasma creatinine *μmol/L*	252 (26)[bc]	50 (6)	54 (5)
Period of uremia/sham uremia *weeks*	14.1 (0.4)	14.3 (0.4)	14.7 (0.4)
Passive diameter of artery at 100 mm Hg *μm*	151 (4)[d]	171 (3)	150 (5)[d]

[a] $P = 0.02$ compared to WKYC
[b] $P < 0.001$ compared to SHRC
[c] $P < 0.001$ compared to WKYC
[d] $P < 0.01$ compared to WKYC

separate groups: WKYU, WKYC, and SHRC. Therefore, comparisons were made using analysis of variance (ANOVA). For single-valued variables such as plasma creatinine, one-way ANOVA was employed. When comparison of a series of measurements, such as the set of values of normalized diameter across the pressure range, was required between groups, then repeated measures ANOVA was chosen [23]. The Bonferroni correction was applied to all analyses, allowing direct comparison between any combination of two groups. The significance level was taken as $P < 0.05$.

RESULTS

Both WKYU and SHRC were significantly hypertensive compared to WKYC. WKYU were anemic, with a lower body mass and higher plasma creatinine values than both WKYC and SHRC (Table 1). The low body weight of WKYU was probably due to uremic anorexia; the pair-fed control animals received a minimum of 20 g/day food, and so their weight was greater than WKYU.

Cremaster arteries from WKYU and SHRC had smaller passive internal diameters when compared to WKYC vessels (Fig. 1). For example, at 100 mm Hg—the pressure selected for morphological comparison between strains in a previous study [21]—both WKYU and SHRC vessels had an average diameter of approximately 20 μm less than WKYC (Table 1).

Ten of 18 (56%) vessels from WKYU, 13 of 19 (68%) from WKYC, and 13 of 14 (93%) from SHRC developed satisfactory myogenic tone, defined here as >10% constriction at 100 mm Hg. Myogenic constriction was not different between groups when the sets of values of active normalized diameter across the pressure range were compared by repeated measures ANOVA (Fig. 2). Inspection of the active diameter-pressure relationship at individual points demonstrated that, beyond 160 mm Hg, WKYC vessels underwent forced dilation whereas WKYU and SHRC were able to maintain substantial constriction (Fig. 1).

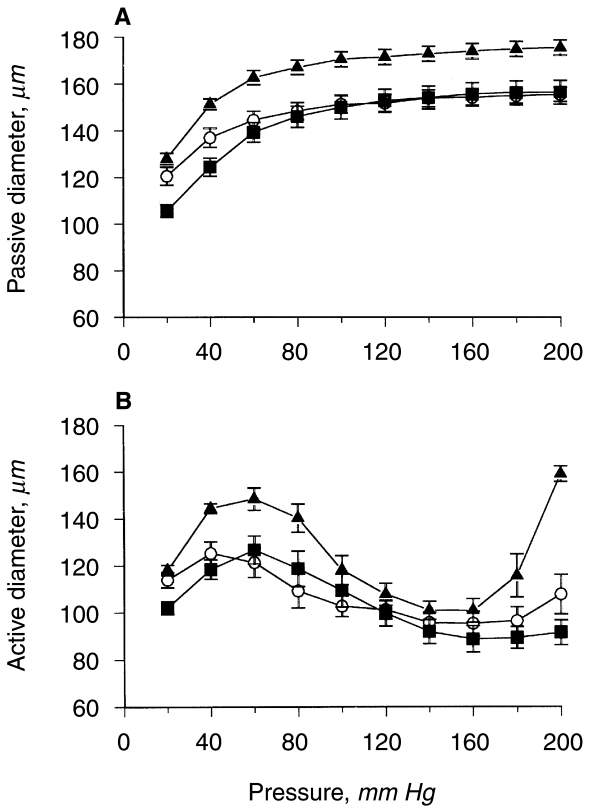

Fig. 1. Passive and active diameters of cremaster arteries plotted against lumen pressure. Symbols are (○) WKYU, $N = 10$; (▲) WKYC, $N = 13$; (■) SHRC, $N = 13$.

DISCUSSION

Uremic hypertension is associated with increased peripheral vascular resistance [15]. Many endogenous agents such as noradrenaline, nitric oxide, and endothelin can influence resistance artery tone [24]. The dominant mechanism responsible for small artery tone in vivo, however, is not clearly established. Studies of isolated pressurized resistance vessels [19, 20] support the notion that myogenic constriction is critically important to basal vascular resistance [18]. Therefore, the purpose of our current study was to establish whether an increased myogenic response could account for uremic hypertension. The results demonstrate that myogenic tone is not increased in skeletal muscle resistance arteries (branch 1A) of uremic hypertensive or spontaneously hypertensive rats.

To our knowledge this is the first description of the myogenic response of a resistance artery in uremia, and we conclude that uremic hypertension is not the result of increased myogenic tone. However, femoral arteries from mildly uremic normotensive animals have shown a small increase in spontaneous constriction compared to controls [25], which was abolished by angiotensin converting enzyme inhibitor therapy [26]. Although the femoral conduit artery (~ 500 μm passive diameter) is not strictly comparable to the cremaster resistance artery

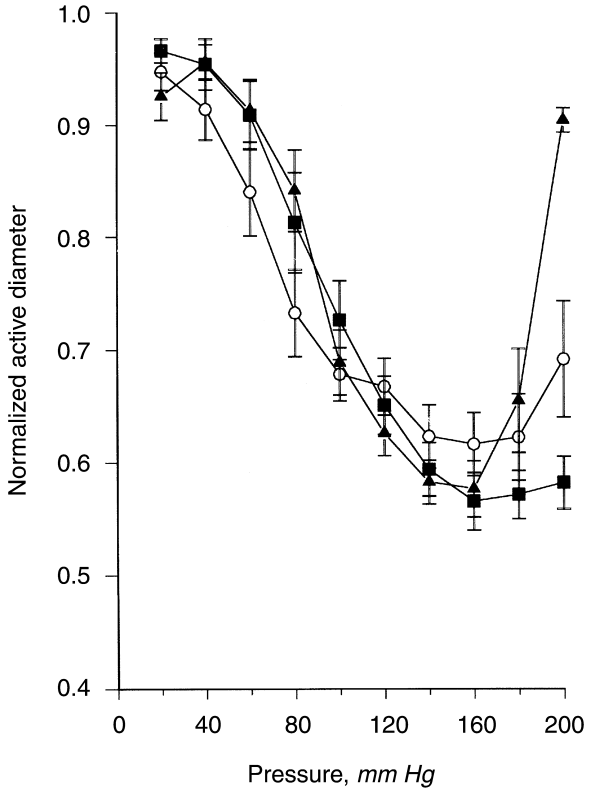

Fig. 2. Normalized active diameter plotted against lumen pressure. Symbols are (○) WKYU, $N = 10$; (▲) WKYC, $N = 13$; and (■) SHRC, $N = 13$). P value not significant between groups by repeated measures ANOVA.

(~ 160 μm passive diameter), the matter deserves consideration. The renin-angiotensin system is activated early in the course of chronic renal failure following partial nephrectomy but, with progression of uremia, sodium retention occurs sufficient to suppress the aberrant renin system [27]. Since vascular renin is mainly derived from uptake of circulating renin of kidney origin [28, 29], and because tissue levels tend to parallel plasma renin activity [30], myogenic responses might be expected to differ in the observed manner as uremia progresses.

If myogenic tone is normal in uremic hypertension, then it follows that increased peripheral resistance occurs by some other means, and several possibilities exist. First, extracellular volume expansion due to inadequate sodium excretion commonly occurs in renal failure [6, 7, 27], raising cardiac output. Resistance vessels respond appropriately with myogenic constriction to autoregulate blood flow [31], and a new equilibrium is established at which cardiac output is near normal but arterial pressure and peripheral resistance are increased [7]. Second, activity of the renin-angiotensin-aldosterone system inappropriate to the prevailing blood pressure occurs in uremia [8, 9], and this increases peripheral resistance through angiotensin II-mediated vasoconstriction. This may represent an abnormal renin response from diseased or scarred [32] kidneys, and consistent with this is the observation that bilateral nephrectomy in dialysis patients causes a lowering of blood pressure and of peripheral resistance [6, 15]. However, in both experimental renal failure [27] and in a proportion of dialysis patients [8], plasma renin activity is not completely autonomous, and can be suppressed by extracellular volume expansion such that inappropriate plasma renin activity and volume expansion do not necessarily co-exist. Third, the sympathetic nervous system is activated in uremia [10, 11], which might increase vascular tone through noradrenaline-induced constriction. Fourth, endothelial dysfunction is well documented in uremia [12, 33]; through lack of endothelium-derived vasodilators, for example nitric oxide, unopposed local and circulating vasoconstrictors such as endothelin might maintain elevated systemic resistance. Finally, structural alterations of resistance arteries occur in uremia [13, 14] similar to those seen in several forms of hypertension [34]. They comprise thickening of the vascular wall and/or narrowing of the lumen, as was apparent in both our WKYU and SHRC vessels, and might impart an increased resistance on small vessels [24]. Therefore, there are several alternative mechanisms to an abnormal myogenic response that are capable of raising peripheral resistance in uremic hypertension. Some of them, such as increased circulating angiotensin, conceivably might enhance the myogenic response to transluminal pressure elevation in vivo, an effect that would not have been observed in isolated vessels.

Our results are consistent with a previous study of spontaneously hypertensive rat arteries [20] in which myogenic tone was increased, compared to control Wistar-Kyoto vessels, only at high lumen pressures. In our study, beyond 160 mm Hg, vessels from SHRC and WKYU were able to maintain constriction whereas forced dilation of WKYC vessels occurred. This was probably a consequence of differing vessel diameters; by Laplace's Law, wall tension is equal to the product of lumen pressure and radius, such that the smaller diameter SHRC and WKYU arteries must have experienced less wall tension than WKYC at a given lumen pressure. However, because the pressure experienced by small (100 to 200 μm) arteries in vivo is between 64% [35] and 80% [16] of the aortic pressure, it is unlikely that the in vitro data obtained beyond 160 mm Hg are physiologically relevant even to SHRC. Our data differ in one respect from other studies that demonstrated increased slope of the normalized active pressure-diameter relationship of cremaster arteries of the spontaneously hypertensive rat [19], which we did not observe (Fig. 2) despite using the same anatomical vessel. The animals we studied were approximately 26-weeks-old at sacrifice, compared to 15- to 20-weeks-old [19]. Since the myogenic response is enhanced in the spontaneously hypertensive rat during the onset of hypertension [20], we may have observed

vessels at a different stage of development. Consistent with this is the observation in skeletal muscle arterioles of similar myogenic properties across the physiological pressure range in approximately 25-week-old spontaneously hypertensive rats compared to normotensive controls [23].

In summary, this study has assessed the myogenic response in isolated resistance arteries from uremic hypertensive animals. No increase in myogenic tone was observed over the physiological pressure range. We conclude that increased myogenic constriction is not responsible for the hypertension of chronic renal failure.

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