Cerebral artery responses to pressure and flow in uremic hypertensive and spontaneously hypertensive rats

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Cerebral artery responses to pressure and flow in uremic hypertensive and spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 284: H1212–H1216, 2003; 10.1152/ajpheart.00644.2002.—Impaired cerebral blood flow autoregulation is seen in uremic hypertension, whereas in nonuremic hypertension autoregulation is shifted toward higher perfusion pressure. The cerebral artery constricts in response to a rise in either lumen pressure or flow; we examined these responses in isolated middle cerebral artery segments from uremic Wistar-Kyoto rats (WKYU), normotensive control rats (WKYC), and spontaneously hypertensive rats (SHR). Pressure-induced (myogenic) constriction developed at 100 mmHg; lumen flow was then increased in steps from 0 to 98 μl/min. Some vessels were studied after endothelium ablation. Myogenic constriction was significantly lower in WKYU (28 ± 2.9%) compared with both WKYC (39 ± 2.5%, P = 0.035) and SHR (40 ± 3.1%, P = 0.018). Flow caused constriction of arteries from all groups in an endothelium-independent manner. The response to flow was similar in WKYU and WKYC, whereas SHR displayed increased constriction compared with WKYU (P < 0.001) and WKYC (P < 0.001). We conclude that cerebral myogenic constriction is decreased in WKYU, whereas flow-induced constriction is enhanced in SHR.

myogenic tone; flow-induced constriction; myograph

UREMIA IS ASSOCIATED WITH STROKE, particularly hemorrhagic stroke, in animals (25) and humans (10). Although raised blood pressure often contributes to this risk (11, 15), other factors may be important. In the stroke-prone hypertensive rat, uremia develops due to hypertensive glomerulosclerosis (9), followed by impaired cerebral autoregulation, before stroke (26, 28). Humans with renal failure have increased cerebral blood flow with decreased vasodilatory capacity (16). Therefore, it is possible that an abnormality of cerebral vascular autoregulation, characterized by inadequate constriction, results from uremia.

Conversely, hypertensive (nonuremic) animals (1, 21) and humans (17, 29) show vascular adaptation with a shift of cerebral blood flow autoregulation toward higher levels of arterial pressure. The increased vascular resistance is largely due to greater constriction, rather than to smooth muscle hypertrophy (23), and maintains constancy of blood flow despite systemic hypertension.

Cerebral vascular resistance is to a major extent determined by the degree of contraction of larger proximal arteries such as the middle cerebral artery (7), unlike other vascular beds, in which most of the resistance to blood flow occurs in arteries below ~200 μm diameter (4, 20). Large artery tone therefore regulates cerebral microvascular pressure (1, 7), and it has been suggested that this mechanism protects more distal wall-thinned intracranial blood vessels (8). If this is the case, then enhanced proximal cerebral vascular resistance may guard against stroke in chronic nonuremic hypertension, whereas inadequate vascular constriction seen in uremic hypertension might predispose to stroke.

Because cerebral artery tone increases in response to a rise in either lumen pressure (22) or flow (2, 18), the changes in cerebral autoregulation in uremic and nonuremic hypertension might conceivably reflect changes in either of these processes. We hypothesized that uremia impairs the response to pressure and/or flow in cerebral arteries. Using Wistar-Kyoto (WKY) rats, we studied isolated middle cerebral artery segments from the subtotal nephrectomy rat model of uremic hypertension (WKYU), comparing them with sham-operated normotensive control vessels (WKYC) and spontaneously hypertensive rat vessels (SHR).

MATERIALS AND METHODS

Animals. All procedures had the prior approval of the Home Office (project license no. 70/5014) and were performed in accordance with the Animals Scientific Procedures Act of 1986. Male WKY and SHR (12–14 wk old) were obtained from Harlan. General anesthesia was induced for both stages of nephrectomy by an intramuscular injection of 1 ml/kg body wt Hypnorm (0.315 mg/ml fentanyl citrate plus 10 mg/ml fluanisone; Janssen-Cilag), followed by an intraperitoneal injection of 2.5 mg/kg diazepam (CP Pharmaceuticals). Postoperative analgesia was provided by subcutaneous injection of buprenorphine (50 μg/kg, Schering-Plough). Left subtotal nephrectomy was performed through a midline abdominal incision, removing approximately three-fourths of the renal mass. A total right nephrectomy was performed through a flank incision 1 wk later. WKYC and SHR animals underwent sham surgery in which the appropriate kidney was removed.
stipped of its capsule only. Animals were maintained for ~3 mo during which WKYU and SHR were pair fed with their WKYU partners. Approximately 5 days before death, animals were placed in a metabolic cage for a timed overnight urine collection. Under halothane anesthesia (Concord Pharmaceuticals), an abdominal aortic catheter (0.61 mm outer diameter and 0.28 mm inner diameter, Sims Portex) was then inserted retrogradely through the femoral artery, hepatized, and exteriorized through the skin at the nape of the neck (13). Two to three days later, conscious blood pressure was measured with a pressure transducer (model 60-3003, Harvard Apparatus). Blood was drawn for analysis, and the animal was euthanized by cervical dislocation. The brain was removed immediately and placed in ice-cold physiological saline solution (PSS). A single artery was studied from each rat on the day of death; the cerebral hemisphere was pinned to a Silastic base within a petri dish containing cold PSS, and the middle cerebral artery was dissected free from surrounding tissue under view of a binocular microscope (Stemi SV6, Zeiss).

**Biomechanical and hematological analysis.** Plasma and urinary creatinine concentrations were determined by the Jaffe rate method with the use of a creatinine analyzer (Beckman Coulter). Blood hemoglobin was measured using a β-hemoglobin photometer (Hemocue).

**Drugs and solutions.** All chemicals (Sigma-Aldrich) were made up on the day of the experiment in sterile distilled AnaLAR water (Merck). PSS was composed of the following (in mmol/l): 119 NaCl, 4.7 KCl, 25 NaHCO3, 1.17 KH2PO4, 1.17 MgSO4, 2.0 CaCl2, and 5.5 glucose. CaCl2 was added to PSS immediately before use. Ca2+-free PSS contained 1 mmol/l EGTA and 100 μmol/l sodium nitroprusside.

**Perfusion myograph.** The method of perfusion myography has been described (14). An artery was mounted between the two glass microcannulas (tip diameter 100 μm) of a myograph (Living Systems Instrumentation, Burlington, VT), aligned with its proximal end to the proximal cannula. Care was taken to ensure that the hydraulic resistances of each cannula, determined by the pressure-flow relationship, were equal. Pressure was measured continuously by transducers at both the proximal and distal cannulas. The vessel was slowly pressurized to 100 mmHg by a pump attached to the proximal cannula and tested for leaks; dissipation of pressure indicated leakage, in which case the vessel was discarded. PSS was gassed (95% O2-5% CO2), heated in a reservoir, and circulated at a rate of 40 μl/min to the vessel chamber, which was continuously monitored and maintained at pH 7.40 and 37°C. The vessel was viewed on the stage of an inverted microscope (model TMS-F, Nikon) through a ×10 objective lens that was video linked to a monitor. Vessel internal diameter was continuously measured by a video dimension analyzer and saved to computer disk. Equilibration at 37°C with zero lumen flow for 1 h resulted in spontaneous development of adequate myogenic tone (>10% constriction) in the vast majority of vessels. Acetylcholine (10−5 M) was added to the bath, and dilation from baseline myogenic constriction of >20% was considered to indicate an intact endothelium. In some experiments, the endothelium was ablated by perfusing water for 1 min. The absence of dilation to 10−5 M acetylcholine after water perfusion was taken as evidence of successful endothelial ablation. Not infrequently, water perfusion had to be repeated to ablate the endothelium. In almost all cases, myogenic constriction of >10% returned after water perfusion, confirming that vascular smooth muscle was still functional. At the end of all experiments, vessels were superfused with Ca2+-free solution, and passive diameter was measured at 100 mmHg.

Flow experiments were performed by increasing lumen flow rate from 0–98 μl/min. Flow rate was controlled by a second pump that was attached to the distal cannula. Increase in the flow rate of PSS out of the distal cannula caused reduction of distal cannula pressure and therefore of mean pressure across the cannulas. To maintain average pressure across both cannulas constant at 100 mmHg, the proximal pressure was increased manually. Because the resistance of each cannula was identical, mean pressure across them was equal to the pressure in the vessel lumen, assuming laminar flow through the system, and so it was possible to alter flow without changing lumen pressure. Flow was continued at each rate for ~3–4 min, until a stable response was obtained.

The perfusate consisted of pregassed PSS, which could potentially lose CO2 tension while traversing the proximal tubing system. For this reason, perfusate passed through a 12-cm-long gas-permeable (silicon) tube submerged in the PSS of the myograph chamber immediately before reaching the proximal cannula. This allowed for reequilibration of CO2 between chamber and perfusate. This would minimize the possibility of inadequately equilibrated perfusate causing cerebral constriction because cerebral artery tone is sensitive to pH (27). Control experiments confirmed that when un-gassed nonbicarbonate buffer was used as perfusate, constriction to flow was observed, further suggesting that flow-induced constriction is due to shear stress and not to alkalinity of the perfusate. Finally, although it is impossible to measure the temperature of the perfusate as it enters the vessel due to the dimensions involved, inadequate heat equilibration would, if present, be expected to cause dilation rather than constriction (5).

**Calculations and statistical analysis.** Wall shear stress (σ; in dyn/cm2) was calculated from the equation \[
\sigma = 4 \eta Q / \pi r^2,
\] where η is the viscosity of PSS taken as 0.007 dyn·s·cm−2 (14), Q is the lumen flow rate (μl/min), and r is the internal radius (μm) of the vessel. Myogenic constriction (MC [%]) was calculated from the equation MC = 100 × (Dp − Da)/Da, where Dp is the passive diameter and Da is the active diameter at 100 mmHg of lumen pressure. Flow-induced constriction (FC [%]) was assessed by the equation FC = 100 × (Dp − Df)/Da, where Df is the diameter with myogenic constriction at zero flow rate and Dp is the diameter at any given flow rate (2). Creatinine clearance [ClCr (ml/min)] was calculated using the equation ClCr = (Uc × V)/(Pc × t), where Uc is urine creatinine, Pc is plasma creatinine concentration (μmol/l), and V is the volume of urine (ml) passed in time t (min).

Values are expressed as the means ± SE. All comparisons were made between three groups: WKYU, WKYC, and SHR. Single-valued variables such as plasma creatinine were compared by one-way ANOVA. A series of measurements, such as the flow-diameter relation, were compared by repeated-measures ANOVA. The Bonferroni correction was applied to all analyses, allowing direct comparison between all combinations of two groups. The significance level was taken as \( P < 0.05 \).

**RESULTS**

General information regarding the animals and the cerebral arteries from each group are given in Table 1. Mean arterial pressure was significantly greater in SHR than in WKYU, and WKYU were significantly hypertensive relative to WKYC. Substantial uremia developed in WKYU.

At 100 mmHg of distending pressure and zero lumen flow, WKYU developed significantly less myogenic con-
striction (28 ± 2.9%) compared with WKYC (39 ± 2.5%, \( P = 0.035 \)) and SHR (40 ± 3.1%, \( P = 0.018 \), \( n = 17 \) per group). From these, flow responses were successfully studied in 10 animals per group, and flow responses after endothelial ablation in 2–4 animals per group. Assessment of flow responses was abandoned in some arteries due to cannula blockage or passage of an air bubble through the vessel lumen.

Vessel diameter, as assessed by repeated-measures ANOVA across the flow range, was significantly greater in WKYU compared with WKYC (\( P < 0.001 \)) and also significantly greater in WKYC compared with SHR (\( P < 0.01 \)) (Fig. 1).

All arteries constricted to increasing lumen flow (Fig. 1). Flow-induced constriction was evident in arteries even after ablation of endothelium (data not shown). WKYU and WKYC showed similar percent changes in diameter to flow (\( P \) not significant), with constriction at low-flow rates and plateau at higher rates (Fig. 2). SHR exhibited enhanced constriction to flow compared with both WKYU (\( P < 0.001 \)) and WKYC (\( P < 0.001 \)), with progressive constriction over almost the entire flow range (Fig. 2). A similar pattern of response was seen when diameter was plotted against shear stress rather than flow rate (Fig. 3).

**DISCUSSION**

The major findings of this study are that cerebral myogenic constriction is impaired in arteries from WKYU, whereas increased constriction to flow is seen in SHR, compared with normotensive controls.

Pressure-dependent (myogenic) constriction is well described in rat cerebral arteries; at 100 mmHg, WKYC and SHR demonstrated equal response in our study, in keeping with a prior comparison (22). The

![Fig. 1](image1.png)

Fig. 1. Cerebral artery diameter plotted against lumen flow (logarithmic scale) in endothelium-intact vessels. ◊, Uremic Wistar-Kyoto rats (WKYU); ●, control Wistar-Kyoto rats (WKYC); ▲, spontaneously hypertensive rats (SHR); \( n = 10 \) rats/group. \( P < 0.01 \) for comparison of WKYU with both WKYC and SHR, whereas \( P < 0.01 \) for comparison of WKYC with SHR.

![Fig. 2](image2.png)

Fig. 2. Data from Fig. 1 represented as the percent of flow-induced constriction plotted against lumen flow (logarithmic scale). ◊, WKYU; ●, WKYC; ▲, SHR; \( n = 10 \) rats/group. \( P < 0.001 \) for comparison of SHR with both WKYU and WKYC.

### Table 1. General data

<table>
<thead>
<tr>
<th></th>
<th>WKYU</th>
<th>WKYC</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>157 ± 8†‡</td>
<td>134 ± 3</td>
<td>196 ± 4‡</td>
</tr>
<tr>
<td>Blood hemoglobin, g/dl</td>
<td>7.5 ± 0.5†‡</td>
<td>13.0 ± 0.5</td>
<td>14.3 ± 0.4</td>
</tr>
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<td>Plasma creatinine, µmol/l</td>
<td>266 ± 41†‡</td>
<td>52 ± 5</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>0.26 ± 0.09†‡</td>
<td>2.40 ± 0.58</td>
<td>2.48 ± 0.41</td>
</tr>
<tr>
<td>Weight, g</td>
<td>290 ± 11†‡</td>
<td>364 ± 6</td>
<td>376 ± 6</td>
</tr>
<tr>
<td>Duration of uremia/sham uremia, wk</td>
<td>14.4 ± 0.5</td>
<td>15.0 ± 0.6</td>
<td>14.4 ± 0.4</td>
</tr>
<tr>
<td>Cerebral artery passive diameter at 100 mmHg, µm</td>
<td>231 ± 8</td>
<td>239 ± 6</td>
<td>225 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 17 \) rats/group. General data for experimental animals and cerebral arteries are shown. WKYU, uremic Wistar-Kyoto rats; WKYC, control Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. †\( P = 0.014 \) compared with WKYC; ‡\( P < 0.005 \) compared with SHR; §\( P < 0.005 \) compared with WKYC.
effect of uremia on cerebral myogenic constriction has previously been studied in nephrectomized SHR and in stroke-prone SHR (25), which develops spontaneous hypertensive glomerulosclerosis as well as stroke. Vessels from these two strains of SHR with renal failure were found to have decreased myogenic tone, determined by their contractile response to a single brief large pressure step. However, such pressure increments, in addition to stimulating myogenic contraction, may elicit transient nitric oxide release from rat cerebral artery endothelium (6) and might therefore underestimate myogenic constriction. Further evaluation of stroke-prone SHR cerebral arteries suggested that levels of myogenic tone were normal (12), although the level of uremia, if present, was not reported. Our study is, therefore, the first to establish the degree of cerebral myogenic constriction in uremia, measured under steady-state conditions, in a normotensive rat strain possessing genetically typical arteries. The finding of substantially decreased tone in WKY compared with WKYC and SHR supports the notion of uremic impairment of myogenic constriction. This might explain the impaired cerebral autoregulation seen in uremia in vivo.

Flow-dependent constriction is known to occur in the rat middle cerebral artery; a previous report (2) established that vessels responded with \( \sim 15\% \) decrease in diameter when exposed to 100 dyn/cm\(^2\) shear stress. This value is very similar to that seen in our WKY and likewise appears to be endothelium independent. The response of cerebral arteries to flow, either in uremia or hypertension, has not hitherto been documented. Our finding of an augmented constriction to flow in SHR is compatible with the observed in vivo pattern of cerebral blood flow autoregulation in SHR (21). If increased constriction to flow is an adaptation to raised blood pressure, rather than a property unique to the SHR cerebral artery, then this adaptation was not present in uremic hypertension.

In addition to the experimental data discussed above, it seems likely, on clinical grounds, that proximal resistance artery inadequacy in the brain results in cerebral overperfusion and microvascular damage. First, in acute hypertensive encephalopathy, there is failure of autoregulatory vasoconstriction with dilation of distal vessels and increased cerebral blood flow (30); fibrinoid necrosis of arterioles leads to cerebral microinfarcts and petechial hemorrhages (3). Second, the cerebral hyperperfusion syndrome may follow cerebral revascularisation by endarterectomy (24) or stent placement (19); newly elevated perfusion pressure overwhelms arteriolar autoregulation, sometimes precipitating intracerebral hemorrhage. Finally, a breakdown of cerebral autoregulation with hyperperfusion may lead to vasogenic cerebral edema in the preeclampsia/eclampsia syndrome (33). It appears reasonable to speculate that dissipation of perfusion pressure early in the course of the cerebral circulation may be necessary to maintain an intact microvasculature; if this fails, and particularly if systemic pressure is high, stroke may occur. Although in (stroke resistant) SHR microvascular pressure is maintained low relative to arterial pressure by a raised cerebral artery resistance (1), similar direct microvascular pressure measurements are lacking in uremia. Such information would be required to confirm whether or not the above reasoning can be applied to uremia.

There was no evidence of stroke in uremic hypertensive rats. This contrasts with the situation in stroke prone SHR. The mean arterial pressure of WKY was raised compared with WKYC, although was still substantially lower than that of SHR. Because lowering blood pressure in stroke-prone SHR is known to prevent stroke (31, 32), it seems likely that both a very high arterial pressure and a deficiency of cerebral autoregulation are required for development of stroke. Therefore, stroke might not be expected to occur in the WKU model.

In conclusion, cerebral arteries from uremic hypertensive animals display impaired myogenic constriction but similar response to flow compared with normotensive controls. Spontaneously hypertensive animals manifest similar myogenic tone yet enhanced constriction to flow compared with normotensive controls. The findings might help explain previous observations of impaired cerebral blood flow autoregulation in uremic hypertension and enhanced autoregulatory vasoconstriction in nonuremic hypertension. These data suggest a possible basis for the association of stroke with uremia in humans.
REFERENCES