Chronic renal failure alters endothelial function in cerebral circulation in mice

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Bugnicourt JM, Da Silveira C, Bengrine A, Godefroy O, Baumbach G, Sevestre H, Bode-Boeger SM, Kielstein JT, Massy ZA, Chillon JM. Chronic renal failure alters endothelial function in cerebral circulation in mice. Am J Physiol Heart Circ Physiol 301: H1143–H1152, 2011. First published June 24, 2011; doi:10.1152/ajpheart.01237.2010.—We examined structure, composition, and endothelial function in cerebral arterioles after 4 wk of chronic renal failure (CRF) in a well-defined murine model (C57BL/6J and apolipoprotein E knockout female mice). We also determined quantitative expression of endothelial nitric oxide synthase (eNOS), phosphorylated eNOS (on serine 1177 and threonine 495), and caveolin-1; quantitative expression of markers of vascular inflammation or oxidative stress [Rock-1, Rock-2, VCAM-1, and peroxisome proliferator-activated receptor-γ (PPARγ)]; and the plasma concentration of l-arginine and asymmetric dimethylarginine (ADMA). Our hypothesis was that endothelial function would be impaired in cerebral arterioles during CRF following either a decrease in NO production (through alteration of eNOS expression or regulation) or an increase in NO degradation (due to oxidative stress or vascular inflammation). Endothelium-dependent relaxation was impaired during CRF, but endothelium-independent relaxation was not. CRF had no effect on cerebral arteriolar structure and composition. Quantitative expressions of eNOS, eNOS phosphorylated on serine 1177, caveolin-1, Rock-1, Rock-2, and VCAM-1 were similar in CRF and non-CRF mice. In contrast, quantitative expression of PPARγ (which exercises a protective role on blood vessels) was significantly lower in CRF mice, whereas quantitative expression of eNOS phosphorylated on the threonine 495 (the inactive form of eNOS) was significantly higher. Lastly, the plasma concentration of ADMA (a uremic toxin and an endogenous inhibitor of eNOS) was elevated and plasma concentration of l-arginine was low in CRF. In conclusion, endothelial function is impaired in a mouse model of early stage CRF. These alterations may be related (at least in part) to a decrease in NO production.

Cardiovascular disease is highly prevalent in patients with chronic renal failure (CRF) and may account for 50% of all deaths in this population (39). Stroke is the third most common cause of cardiovascular death in CRF sufferers. Patients with end-stage renal disease (ESRD) have a 4- to 10-fold greater risk of hospitalized ischemic and hemorrhagic stroke (35), an increased risk of cognitive impairment and dementia (28, 36), and a poor long-term poststroke prognosis (13) compared with non-ESRD individuals. Furthermore, the prevalence of asymptomatic, silent, cerebral infarction is four to five times higher in dialysis patients than in age- and gender-matched controls (29). Moreover, patients on dialysis with cognitive impairment appear to have a high number of cortical defects that are reminiscent of multiple infarct-related damage (20).

The higher frequency of stroke and cognitive impairment in ESRD patients cannot be solely explained by their higher prevalence of traditional (27) and nontraditional risk factors (17). Other CRF-related factors (such as the accumulation of uremic toxins or arterial calcification) may account for the alteration in cerebral perfusion. Indeed, uremic toxins like asymmetric dimethylarginine (ADMA) may accelerate atherosclerosis or lead to cerebral vasocostriction (16). Furthermore, atherosclerosis and arteriosclerosis are frequently comorbid and, in patients with ESRD, are characterized by a high degree of both intimal and medial calcification. Indeed the extent of calcification and the degree of arterial stiffening are independent predictors of mortality (21). However, there are few data on cerebral perfusion, cerebral blood vessel structure, and the impact of uremic toxins and arterial calcification in CRF.

We decided to examine the impact of CRF on cerebral arteriolar structure and function in a well-defined model of CRF in two mouse strains with the same genetic background, C57 black wild-type (WT) and apolipoprotein gene knockout (ApoE−/−) female mice (26). The rationale for using female mice and two different strains is based on the observation that male and female WT mice do not develop calcification during CRF. In contrast, ApoE−/− mice develop arterial calcification during CRF (24), but the process is much slower in males than in females. Since vascular calcification may affect vascular structure and function, we wondered whether this would be true for the cerebral circulation.

The present study’s first objective was to examine cerebral arteriolar endothelium-dependent relaxation in our murine CRF model. We hypothesized that endothelium-dependent relaxation would be impaired in cerebral arterioles, as previously observed in the aorta (24).

The second objective was to evaluate cerebral arteriolar structure and composition in the CRF model. In view of our observation that endothelial dysfunction in the aorta results in an increase in aortic rigidity, we hypothesized that endothelial dysfunction would be accompanied by alterations in the structure and stiffness of cerebral arterioles (24).

The study’s third objective was to investigate the potential mechanisms of endothelial dysfunction in cerebral arterioles.
during CRF. Our hypothesis was that endothelial dysfunction would be related to either a decrease in nitric oxide (NO) production or an increase in NO degradation. To investigate mechanisms potentially responsible for a decrease in NO production, we evaluated the expression of endothelial NO synthase (eNOS), the active form of eNOS (eNOS phosphorylated on serine 1177), the inactive form of eNOS (eNOS phosphorylated on threonine 495), and caveolin-1 (which inhibits eNOS activity by direct protein interaction; Ref. 15). To investigate mechanisms potentially responsible for an increase in NO degradation, we examined markers of vascular inflammation and oxidative stress [Rock-1, Rock-2, VCAM-1, and peroxisome proliferator-activated receptor-γ (PPARγ)]. Lastly, we also assayed the plasma concentrations of ADMA and l-arginine. As mentioned above, ADMA is a uremic toxin and endogenous eNOS inhibitor that accumulates during CRF and may accelerate atherosclerosis or lead to cerebral vasocostriction (16).

METHODS

Animals and Diet

All experiments were performed on female mice purchased from Charles River Laboratories (Lyon, France). The animals were housed in polycarbonate cages in temperature- and humidity-controlled rooms with a 12:12-h light-dark cycle and were given standard chow (Harlan Teklad Global Diet 2018; Harlan, Bicester, UK) and tap water ad libitum. Animals were handled in accordance with French legislation, and the protocol was approved by the Institutional Animal Care Committee, Comité Régional d’Éthique en Matière d’Expérimentation Animale de Picardie.

CRF Induction and Experimental Procedures

WT and ApoE<sup>−/−</sup> mice were randomly assigned to CRF or non-CRF induction groups, i.e., WT mice without CRF (non-CRF WT), WT mice with CRF (CRF WT), ApoE<sup>−/−</sup> mice without CRF (non-CRF ApoE<sup>−/−</sup>), and ApoE<sup>−/−</sup> mice with CRF (CRF ApoE<sup>−/−</sup>). To induce CRF, we applied cortical electrocautery to the right kidney in 8-wk-old mice and then performed 2 wk later left total nephrectomy in 10-wk-old mice. The mice were anesthetized with an intraperitoneal injection of 80 mg/kg ketamine and 8 mg/kg xylazine (26). The short duration of surgery meant that there was no need for subsequent administration of anesthetics. Animals received a subcutaneous administration of buprenorphine (1.5 mg/kg) for postsurgery pain control. Control animals underwent a intraperitoneal injection of 80 mg/kg ketamine and 8 mg/kg xylazine. Anesthesia was supplemented with pentobarbital (5 mg/100g) via a catheter introduced into the left femoral artery whenever paw pressure evoked a change in blood pressure or heart rate. The adequacy of anesthesia was evaluated every 10 min. Mice were ventilated, arterial blood pressure was monitored, and arteriolar diameter was measured using a cranial window (3). Body temperature was maintained at ~37°C with a heating pad. Arteriolar pressure and diameter on the cerebrum were measured as described previously (3).

Measurement of Arteriolar Diameter

We measured the internal diameter of first-order cerebral arterioles by using an open-skull technique (3). The head was placed in an adjustable head holder, and the skull was exposed by a 1-cm skin incision. A dam of dental acrylic was constructed around the exposed skull, and ports were placed for inflow and outflow of artificial cerebrospinal fluid (CSF). Craniotomy was performed over the left parietal cortex, and the dura was incised to expose cerebral blood vessels. The exposed brain was continuously suffused with artificial CSF, warmed to 37–38°C, and equilibrated with a gas mixture of 5% CO<sub>2</sub>-95% N<sub>2</sub>. The composition (in mmol/l) of the CSF was as follows: 3.0 KCl, 0.6 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub>, 131.9 NaCl, 24.6 NaHCO<sub>3</sub>, 6.7 urea, and 3.7 glucose. Arteriolar diameter was monitored with a microscope (Stemi 2000-C; Carl Zeiss, Jena, Germany) connected to a closed-circuit video system. The final magnification was ×298. Images were digitized with a video frame grabber, and the diameter was measured using image analysis software (Saisam; Microvision Instruments, Evry, France).

Measurement of Cerebral Arteriolar Pressure

Cerebral arteriolar pressure was measured with a micropipette connected to a servo-null pressure-measuring device (model 5A; Vista Electronics, Ramona, CA). Pipettes were sharpened to a beveled tip (diameter: 3–5 μm), filled with 1.5 mmol/l sodium chloride solution, and inserted into the lumen of a cerebral arteriole using a micromanipulator (3).

Experimental Protocols

Thirty minutes after the completion of surgery, the cerebral arteriolar diameter was measured under baseline conditions. Several protocols were then performed in different groups of mice.

Cerebral arteriolar vasoreactivity. We examined the cerebral arteriolar vasodilatation induced by the endothelium-independent vasodilator sodium nitroprusside (SNP; 10<sup>−7</sup> and 10<sup>−5</sup> M), the endothelium-dependent and receptor-dependent vasodilators acetylcholine (Ach; 10<sup>−6</sup> and 10<sup>−5</sup> M) and adenosine diphosphate (ADP; 10<sup>−7</sup> and 10<sup>−4</sup> M), an endothelium-dependent and receptor-independent vasodilator calcium ionophore (A23187; 10<sup>−7</sup> and 10<sup>−6</sup> M), and a NADPH oxidase substrate (NADPH; 10<sup>−6</sup> and 10<sup>−5</sup> M). Systemic pressure and arteriolar internal diameter were measured before and after 5 min of drug perfusion into the cranial window with CSF vehicle.

Cerebral arteriolar mechanics and structure. After the measurement of cerebral arteriolar pressure and baseline arteriolar internal diameter, cerebral vessels were suffused with artificial CSF containing EDTA (67 mmol/l). This concentration of EDTA is known to completely deactivate vascular smooth muscle in the cerebral arterioles elsewhere (2). The maximum vasodilator response was measured and the cerebral arteriolar pressure-diameter relationship was determined over the mean arteriolar pressure range from 40 to 10 mmHg in 10-mmHg steps. The arteriolar diameter reached a steady state within 15 s of each pressure drop, and the cerebral arteriolar pressure and internal diameter were measured at 30 s later.
After the final pressure step, blood was rein infused to restore pressure to control levels. Suffusion of cerebral vessels with EDTA-containing artificial CSF was discontinued, and the maximally dilated arterioles were fixed by suffusion with glutaraldehyde (2.25% vol/vol in 0.10 mol/l cacodylate buffer). Arterioles were considered to be adequately fixed when blood flow through the arteriole had ceased.

The animal was killed via a pentobarbitone sodium overdose, and the arteriolar segment used for pressure-diameter measurements was removed with a microsurgery knife, processed for electron microscopy, and embedded in Spurr low-viscosity resin while maintaining the cross-sectional orientation. The cross-sectional area (CSA) of the wall was determined histologically from 1-μm thick sections using the video image analyzing system described above. The luminal and total (lumen plus vessel wall) CSAs of the arteriole were measured by tracing the inner and outer edges of the vessel wall. Wall CSA was calculated by subtracting the luminal CSA from the total CSA. Circumferential stress, circumferential strain, tangential elastic modulus, wall thickness, wall thickness-to-lumen ratio, and external diameter were calculated as previously described in detail (2).

Wall composition. The volume density (%) of smooth muscle, elastin, collagen, basement membrane, and endothelium was quantitated from electron micrographs of the vessel wall by using a previously described method (4). Ultrathin sections of the arterioles were cut on a Reichert Ultracut microtome (Cambridge Instruments, Cambridge, NY) and contrasted with phosphotungstic acid (0.25%). Sections were examined with a Hitachi 7000 electron microscope (Hitachi Instruments, Danbury, CT). Electron micrographs were taken at a standard magnification of ×9,000 and enlarged by a factor of 3 to yield a final magnification of ×27,000. To ensure uniform sampling, the vessel was divided into four equal quadrants. Two or three electron micrographs were taken randomly in each quadrant to yield a total of 9 or 10 electron micrographs per section. A standard point counting grid (the double-square lattice test system) was used to count the number of points contained within the profiles of smooth muscle, elastin, collagen, basement membrane, and endothelium. The volume density (Vv) of each component was calculated from the number of points in each component (Pv) and the total number of points contained within the vessel wall (Py): Vv = Pp/Py.

Western blotting. Mouse cerebral microvessels were isolated according to a technique previously described (6). The brain microvessels were homogenized in cold RIPA buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% SDS, 1% deoxycholate, and 1% Triton X100) containing a protease inhibitor cocktail (Complete Mini, Roche Molecular Biochemicals, Indianapolis, IN). Proteins were precipitated with 4 vol of absolute acetone and resuspended in 1× Laemmli buffer. The proteins were separated by 10% SDS-PAGE. After transfer to polyvinyl difluoride membranes, the blots were incubated either with a 1:500-diluted mouse anti-eNOS monoclonal antibody (Abcam, Cambridge, MA), a 1:1,000-diluted rabbit anti-phosphorylated eNOS (serine 1177 or threonine 495) polyclonal antibody (Cell Signaling; Ozyme, St. Quentin en Yvelines, France), a 1:5,000-diluted mouse anti-caveolin-1 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), a 1:500-diluted goat anti-Rock-1 polyclonal antibody (Santa Cruz Biotechnology), a 1:2,500-diluted goat anti-Rock-2 polyclonal antibody (Santa Cruz Biotechnology), a 1:500-diluted rabbit anti-PPARY polyclonal antibody (Santa Cruz Biotechnology), a 1:1,000-diluted goat anti-VCAM-1 polyclonal antibody (Santa Cruz Biotechnology), or a 1:5,000-diluted mouse anti-β-actin monoclonal antibody (Sigma Chemical, St. Louis, MO). All the blots were incubated with the primary antibody overnight at 4°C. The blots were incubated for 1 h at room temperature in the presence of peroxidase-conjugated secondary antibodies (anti-goat, anti-rabbit, or anti-mouse immunoglobulin G from Santa Cruz Biotechnology). The bound antibodies were detected using an enhanced chemiluminescent immunoblotting detection system and X-ray films (GE Healthcare Amersham, Orsay, France). Protein band signals were quantitated using an image-scanning densitometer, and the strength of each band signal was normalized to the corresponding β-actin signal.

Substances Used

Urea, glucose, TRI Reagent, glutaraldehyde, cacodylate, SNP, ADP, A23187, NADPH, KCl, MgCl2, CaCl2, NaCl, and NaHCO3, were purchased from Sigma Chemical. Spurr low-viscosity resin was purchased from Euromedex (Mundolsheim, France). Pentobarbitone sodium, ketamine, and xylazine were purchased from Centravet (Dinan, France).

Statistical Analysis

Results are expressed as means ± SE. Data were examined in a two-way ANOVA that took into account the animal’s status (presence or absence of ApoE−/−, presence or absence of CRF, and the interaction between ApoE−/− and CRF). The threshold for statistical significance was set to P ≤ 0.05. All statistical analyses were performed using Statview software (SAS Institute, Cary, NC).

RESULTS

Serum Biochemistry

Serum urea levels were significantly higher in CRF mice than in non-CRF animals. As expected, serum total cholesterol was significantly higher in ApoE−/− mice. Chronic renal failure enhanced serum total cholesterol levels in WT and ApoE−/− mice but a significantly greater increase was seen in ApoE−/− mice (Table 1).

Table 1. Effects of 4 wk of chronic renal failure on serum urea and total cholesterol levels, body weight, mean arterial pressure, pulse pressure, mean cerebral anteriolar pressure, cerebral anteriolar pulse pressure, and internal diameter in WT and ApoE−/− mice

<table>
<thead>
<tr>
<th>Serum urea, mM</th>
<th>Non-CRF WT</th>
<th>CRF WT</th>
<th>Non-CRF ApoE−/−</th>
<th>CRF ApoE−/−</th>
<th>Effect of ApoE−/−/CRF/Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum TC, mM</td>
<td>2.07 ± 0.03 (62)</td>
<td>2.70 ± 0.06 (56)</td>
<td>6.70 ± 0.32 (51)</td>
<td>12.74 ± 0.44 (52)</td>
<td>0.0001/0.20</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>21.4 ± 0.2 (47)</td>
<td>19.9 ± 0.2 (41)</td>
<td>21.6 ± 0.2 (45)</td>
<td>20.5 ± 0.2 (44)</td>
<td>0.09/0.0001/0.30</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>63 ± 1 (48)</td>
<td>59 ± 1 (40)</td>
<td>61 ± 1 (45)</td>
<td>59 ± 1 (44)</td>
<td>0.97/0.005/0.23</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>29 ± 1 (48)</td>
<td>33 ± 1 (40)</td>
<td>28 ± 1 (45)</td>
<td>30 ± 1 (44)</td>
<td>0.0001/0.001/0.20</td>
</tr>
<tr>
<td>MAPP, mmHg</td>
<td>41 ± 2 (16)</td>
<td>34 ± 2 (11)</td>
<td>36 ± 2 (12)</td>
<td>38 ± 2 (14)</td>
<td>0.97/0.02/0.01</td>
</tr>
<tr>
<td>PPar, mmHg</td>
<td>16 ± 1 (16)</td>
<td>20 ± 2 (11)</td>
<td>14 ± 1 (12)</td>
<td>18 ± 1 (14)</td>
<td>0.07/0.005/0.67</td>
</tr>
<tr>
<td>Baseline ID, μm</td>
<td>44 ± 1 (48)</td>
<td>40 ± 1 (41)</td>
<td>46 ± 1 (45)</td>
<td>46 ± 1 (43)</td>
<td>0.0003/0.15/0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = no. mice). WT, wild-type C57BL/6J mice; ApoE−/−, ApoE−/− gene knockout mice; CRF, chronic renal failure; TC, total cholesterol; MAP, mean arterial pressure; PP, arterial pulse pressure; MAPP, mean cerebral anteriolar pressure; PPar, cerebral anteriolar pulse pressure; ID, internal diameter.
Baseline Parameters

Body weight was significantly lower in CRF mice than in non-CRF animals (Table 1). Mean arterial systemic pressure was significantly lower in CRF mice, whereas pulse pressure was significantly higher (Table 1). Baseline arteriolar pulse pressure was also significantly elevated by CRF (Table 1). Baseline diameter was significantly greater in ApoE/−/−/−/−, compared with WT mice. CRF had no significant effect on baseline diameter (Table 1).

Cerebral Arteriolar Vasoreactivity

Vasodilatation induced by SNP was similar in all four groups (Fig. 1). Acetylcholine-induced vasodilatations were lower in CRF mice, compared with non-CRF mice. These decreases were significant for the highest dose of acetylcholine (10−5M; Fig. 1). At a dose of 10−5 M, ADP-induced vasodilatation was lower in CRF mice than in non-CRF mice. Vasodilatation induced by the highest dose of ADP (10−4 M) was lower in ApoE−/− mice than in WT mice and lower in CRF WT mice than in non-CRF WT mice but was similar in CRF and non-CRF ApoE−/− mice (Fig. 1). In contrast to what was observed for ADP, vasodilatation induced by A23187 was greater in ApoE−/− mice than in WT mice. The vasodilatation induced by A23187 at a dose of 10−6 M was also less intense in CRF mice than in non-CRF mice (Fig. 1). In contrast, the four groups did not differ significantly in terms of NADPH-induced vasodilatation (results not shown).

Mechanics, Structure, and Composition

In deactivated cerebral arterioles, the internal diameter, external diameter, and CSA were significantly greater in ApoE−/− mice than in WT mice, whereas the tangential elastic modulus was significantly lower. In contrast, wall thickness and the wall thickness-to-lumen ratio were similar in WT and ApoE−/− mice. At this age, CRF had no effect on the mechanics and structure of cerebral arterioles in WT and ApoE−/− mice. The volume densities of the vessel wall components were similar in all groups (Table 2 and Fig. 2).

Western Blotting

The quantitative expression of eNOS, eNOS phosphorylated on serine 1177, and caveolin-1 in isolated brain microvessels did not significantly differ in ApoE−/− vs. WT mice or in CRF vs. non-CRF mice. In contrast, quantitative expression of eNOS phosphorylated on threonine 495 was significantly higher in CRF mice than in non-CRF mice and significantly higher in ApoE−/− mice than in WT mice (Fig. 3).
Quantitative expression of Rock-1 and Rock-2 were significantly greater in ApoE<sup>−/−</sup>/H<sup>11002</sup>/H<sup>11002</sup> than in WT mice but did not significantly differ when CRF mice and non-CRF mice are compared. The quantitative expression of VCAM-1 was similar in all four groups of mice. In contrast, quantitative expression of PPARγ was significantly lower in ApoE<sup>−/−</sup>/H<sup>11002</sup>/H<sup>11002</sup> mice than in WT mice and significantly lower in CRF mice than in non-CRF mice (for both WT and ApoE<sup>−/−</sup> animals) (Fig. 4).

Serum ADMA and L-arginine Levels

Serum ADMA levels were significantly elevated by CRF in both WT and ApoE<sup>−/−</sup> mice. In contrast, serum L-arginine

### Table 2. Effects of 4 wk of chronic renal failure on internal diameter, external diameter, cross-sectional area, wall thickness, wall thickness-to-lumen ratio, and tangential elastic modulus measured in fully deactivated (EDTA) cerebral arterioles in WT and ApoE<sup>−/−</sup> mice

<table>
<thead>
<tr>
<th>Vascular structure</th>
<th>nonCRF WT</th>
<th>CRF WT</th>
<th>nonCRF ApoE&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>CRF ApoE&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Effect of ApoE&lt;sup&gt;−/−&lt;/sup&gt;/CRF/Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA ID, μm</td>
<td>62 ± 4 (6)</td>
<td>54 ± 2 (8)</td>
<td>64 ± 2 (9)</td>
<td>64 ± 2 (9)</td>
<td>0.030/0.14/0.12</td>
</tr>
<tr>
<td>EDTA ED, μm</td>
<td>67 ± 4 (6)</td>
<td>59 ± 2 (8)</td>
<td>70 ± 2 (9)</td>
<td>69 ± 2 (9)</td>
<td>0.020/0.12/0.15</td>
</tr>
<tr>
<td>CSA, μm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>470 ± 46 (6)</td>
<td>413 ± 32 (8)</td>
<td>584 ± 36 (9)</td>
<td>518 ± 45 (9)</td>
<td>0.01/0.14/0.91</td>
</tr>
<tr>
<td>WTh, μm</td>
<td>2.3 ± 0.1 (6)</td>
<td>2.3 ± 0.2 (8)</td>
<td>2.8 ± 0.1 (9)</td>
<td>2.4 ± 0.1 (9)</td>
<td>0.08/0.33/0.27</td>
</tr>
<tr>
<td>WTh/l</td>
<td>0.038 ± 0.003 (6)</td>
<td>0.044 ± 0.006 (8)</td>
<td>0.044 ± 0.002 (9)</td>
<td>0.038 ± 0.002 (9)</td>
<td>0.98/0.95/0.08</td>
</tr>
<tr>
<td>TEM</td>
<td>8.2 ± 1.0 (6)</td>
<td>9.5 ± 0.8 (8)</td>
<td>6.9 ± 0.6 (9)</td>
<td>7.2 ± 0.6 (9)</td>
<td>0.02/0.31/0.52</td>
</tr>
<tr>
<td>Vascular composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle cell, %</td>
<td>51.9 ± 3.2 (5)</td>
<td>55.1 ± 2.1 (5)</td>
<td>55.2 ± 1.1 (6)</td>
<td>54.5 ± 2.9 (5)</td>
<td>0.58/0.60/0.43</td>
</tr>
<tr>
<td>Endothelial cell, %</td>
<td>25.4 ± 3.2 (5)</td>
<td>26.4 ± 4.7 (5)</td>
<td>26.4 ± 3.2 (6)</td>
<td>25.5 ± 2.1 (5)</td>
<td>0.99/0.99/0.78</td>
</tr>
<tr>
<td>Elastin, %</td>
<td>15.2 ± 2.6 (5)</td>
<td>11.1 ± 2.6 (5)</td>
<td>10.8 ± 2.7 (6)</td>
<td>12.7 ± 1.6 (5)</td>
<td>0.58/0.66/0.23</td>
</tr>
<tr>
<td>Basement membrane, %</td>
<td>7.2 ± 0.8 (5)</td>
<td>7.3 ± 0.9 (5)</td>
<td>7.6 ± 0.4 (6)</td>
<td>7.2 ± 0.6 (5)</td>
<td>0.90/0.81/0.72</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = no. of mice). ED: external diameter; CSA, cross-sectional area; WTh, wall thickness; WTh/l, wall thickness-to-lumen ratio; TEM, tangential elastic modulus. ID, ED, WTh, and WTh/l were measured or calculated for a mean arteriolar pressure step of 20–30 mmHg.

Quantitative expression of Rock-1 and Rock-2 were significantly greater in ApoE<sup>−/−</sup> than in WT mice but did not significantly differ when CRF mice and non-CRF mice are compared. The quantitative expression of VCAM-1 was similar in all four groups of mice. In contrast, quantitative expression of PPARγ was significantly lower in ApoE<sup>−/−</sup> mice than in WT mice and significantly lower in CRF mice than in non-CRF mice (for both WT and ApoE<sup>−/−</sup> animals) (Fig. 4).

Serum ADMA and L-arginine Levels

Serum ADMA levels were significantly elevated by CRF in both WT and ApoE<sup>−/−</sup> mice. In contrast, serum L-arginine levels were not significantly different between CRF and non-CRF mice of either strain.

**Fig. 2.** Electronic microscopy images in non-CRF WT (A), CRF WT (B), non-CRF ApoE<sup>−/−</sup> (C), and CRF ApoE<sup>−/−</sup> (D) mice. Electron micrographs were taken at a standard magnification of ×9000 and enlarged by a factor of 3 to yield a final magnification of ×27,000. E, elastin; L, lumen; SMC, smooth muscle cell. *Endothelial cell.
levels were significantly lower in CRF mice than in non-CRF mice and this decrease was significantly greater in WT mice. L-arginine levels were also significantly lower in ApoE/H11002/H11002 mice than in WT mice (Fig. 5).

**DISCUSSION**

There are several new findings in the present study. First, endothelium-dependent relaxation is impaired after 4 wk of CRF in both WT and ApoE/H11002/H11002 mice. The alterations in endothelium-dependent vasodilatation were not related to the smooth muscle cells’ inability to respond to NO, since the responses to the NO donor SNP were similar in all four groups. Second, the alterations in cerebral arteriole reactivity were not accompanied by changes in arteriolar structure, mechanics, and composition. Given that endothelium-dependent vasodilatation was impaired in WT and ApoE/H11002/H11002 mice with CRF, it is unlikely that a systemic effect of arterial calcification has a role in these alterations because WT mice with CRF do not develop arterial calcifications (24). Third, the alterations in endothelium-dependent relaxation were not related to any marked...
quantitative changes in the expression of eNOS, the active form of eNOS, caveolin-1, and markers of vascular inflammation and oxidative stress. In contrast, expression level of the inhibited form of eNOS was significantly elevated and expression level of PPARγ (which exercises a protective role on blood vessels) was significantly lower in CRF mice. Fourthly, the alterations in NO-dependent relaxation may also be due to an accumulation of ADMA (a uremic toxin and an endogenous inhibitor of eNOS) and a decrease in the plasma concentration of L-arginine.

A systemic alteration in endothelium-dependent vasodilatation in CRF has already been described (24, 31, 37). We examined endothelium-dependent vasodilatation in cerebral arterioles in our CRF mouse model by using Ach, ADP, and the calcium ionophore A23187. Although the action of these three agents is endothelium dependent (11), the endothelium-derived factors involved differ. Ach is eNOS dependent, and Ach-induced vasorelaxation involves NO release. In cerebral arterioles, responses to ADP are both eNOS dependent and eNOS independent, with the latter mechanism probably being mediated by an endothelium-derived hyperpolarizing factor (10). In contrast to what has been shown in conductance vessels (11), the endothelium-dependent mechanism for cerebral arteriole dilatation by A23187 is cyclooxygenase dependent and may

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**Fig. 4.** Expression of Rock-1 (A), Rock-2 (B), VCAM-1 (C), and peroxisome proliferator-activated receptor-γ (PPARγ; D) in isolated brain microvessels in non-CRF WT, CRF WT, non-CRF ApoE−/−, and CRF ApoE−/− mice. Values are means ± SE. Rock-1 and Rock-2: n = 12 per group and VCAM-1 and PPARγ: n = 8 per group. For Rock-1 (A) and Rock-2 (B), control blots are appropriate as the same loading control was used twice. Blot was first probed with the anti-β-actin antibody, and secondly it was stripped and reprobed with an antibody directed against Rock-1 and then stripped again and reprobed with an antibody directed against Rock-2. *P ≤ 0.05, ApoE−/− vs. WT. †P ≤ 0.05, CRF vs. non-CRF.
involve the release of prostaglandins (32) and superoxide anions radicals (as is observed with bradykinin, another endothelium-dependent and cyclooxygenase-dependent dilator; Ref. 19). Thus the observed alteration in the responses to Ach, ADP, and A23187 suggests that CRF impairs several endothelium-dependent mechanisms.

Several mechanisms may explain the observed decrease in NO-dependent vasodilatation. First, a decrease in NO availability may result from either a decrease in NO production or an increase in NO degradation. It has already been suggested that the decrease in NO production (40) prompted by an accumulation of endogenous eNOS inhibitor(s) (12) may contribute to the decrease in endothelium-dependent dilatation during CRF. Our results are partly in agreement with the latter observations, since our CRF mice showed significantly elevated levels of the endogenous eNOS inhibitor ADMA. Hence, this result suggests that our murine CRF model’s NO production capacity was lowered by accumulation of the endogenous eNOS inhibitor ADMA. This is supported by ADMA’s known ability to constrict human cerebral arteries in vitro (34) and thus decrease the cerebral blood flow in humans in vivo (16). Conversely, overexpression of dimethylarginine dimethylaminohydrolase (the main ADMA-degrading enzyme) inhibits ADMA-induced endothelial dysfunction in the cerebral circulatory system (9). However, our results also suggest that other mechanisms may contribute to the decrease in endothelium-dependent relaxation, since 1) the quantitative expression of eNOS phosphorylated on threonine 495 (the inactive form of eNOS) was significantly elevated in CRF mice, and 2) we observed a significant decrease in L-arginine concentration. Decreased availability of L-arginine has already been described in CRF and may be related to perturbed renal biosynthesis, inhibition of transport in endothelial cells, or greater degradation by other metabolic pathways (5).

A second mechanism that may explain the decrease in endothelial reactivity observed in CRF mice is an increase in oxidative stress and vascular inflammation. However, in CRF mice, vasodilatation induced by stimulation of cyclooxygenase and NADPH oxidase (both enzymes producing reactive oxygen species) with A23187 and NADPH, respectively (8, 18), was either lower than or similar to that observed in non-CRF mice. Furthermore, we did not observe any significant alterations in the quantitative expression of markers of vascular inflammation or oxidative stress, such as Rock-1, Rock-2, and VCAM-1. In contrast, quantitative expression of PPARγ was depressed in CRF mice. PPARγ plays a critical role in protecting blood vessels (33), and interference with PPARγ signaling produces cerebral arteriolar endothelial dysfunction via a mechanism involving oxidative stress. The end result is cerebral vascular hypertrophy and inward remodeling (7). Thus the decrease in endothelial function in cerebral arterioles may result from a decrease in vasoprotective mechanisms rather than an increase in oxidative stress or inflammatory status.

A final possibility is that endothelial dysfunction results from an accumulation of additional, vasoconstrictive uremic toxins [such as diadenosine polyphosphates (38) or angiotensin-A, a structural variant of angiotensin (14, 38)] during CRF. It is well known that the vascular system in ESRD patients undergoes remodeling, in which dilatation and arterial intima-media hypertrophy is associated with arterial stiffening (22, 23). In a CRF rat model, a pressure-independent increase in wall thickness in small intramyocardial arterioles, mesenteric arterioles, and veins has been reported (1). Lastly, another report (30) in a CRF rat model showed that cremaster and mesenteric resistance arteries undergo predominantly eutrophic inward remodeling with uremic hypertension. We did not observe any alteration in the structure, mechanics, and composition of cerebral arterioles in our murine model of CRF. This discrepancy cannot be explained by the absence of increase in pressure in our CRF model, as several of the structural alterations described in other models were pressure-independent. One potential explanation may relate to differences in the animal model used (i.e., mouse vs. rat). A second explanation may involve the duration of CRF. We performed our experiments after 4 wk of CRF, whereas most authors examine vascular structure after 8 wk. However, in one study, alterations in intramyocardial arteriole structure were observed after just 2 wk of CRF (1). It is possible that cerebral arteriolar structural alterations may need more time to develop or that CRF simply does not induce structural or mechanical alterations in cerebral arterioles. Our results are in agreement with previous work in our laboratory (24) using the same murine CRF model that indicated that aortic stiffness was related to endothelial dysfunction rather than structural changes in the aorta.

Fig. 5. Serum asymmetric dimethylarginine (ADMA) levels and L-arginine levels in non-CRF WT, CRF WT, non-CRF ApoE−/−, and CRF ApoE−/− mice. Values are means ± SE; n = 10 per group. *P ≤ 0.05, ApoE−/− vs. WT. †P ≤ 0.05, CRF vs. non-CRF. ‡P ≤ 0.05, interaction.
Our study presents one important limitation, as we did not monitor blood gases during our experiments. However, we have measured blood gases in preliminary experiments in mice with the same body weight as the ones obtained in our CRF and non-CRF WT and ApoE−/− mice. With the same respirator settings (volume and rate) used for our study, blood gases were in the physiological range in the preliminary experiments. In addition, we kept the experiments short to avoid any physiological degradation of the animals during the session and the blood pressure was stable during the experiments. The stability of blood pressure precludes a possible alteration in blood gases, since any alteration in blood gases in our experience leads to a change in blood pressure. Furthermore, if variation in blood gases were responsible for the decrease in vasodilatation observed in CRF mice, we could expect that such a decrease in vasodilatation would be observed for non-CRF mice and for pharmacological agents such as SNP. However, we cannot rule out definitively that period of hypocaenia that is known to limit cerebral artery dilation to stimuli could be responsible for the decreases in vasodilatation observed in our study, and further studies are necessary to clarify this issue.

In conclusion, we observed a decrease in cerebral arteriole endothelium-dependent vasodilatation in a CRF mice model in the absence of any changes in arteriolar structure, mechanics, or composition. These alterations in endothelium-dependent relaxation may be related (at least in part) to a decrease in NO production capacity via an increase in the levels of endogenous eNOS inhibitors (such as ADMA) or an increase in the inactive or composition. These alterations in endothelium-dependent cerebral arterioles in response to bradykinin. Am J Physiol Heart Circ Physiol 280: H2023–H2029, 2001.


