Middle cerebral artery alterations in a rat chronic hypoperfusion model

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Márquez-Martín A, Jiménez-Altayó F, Dantas AP, Caracuel L, Planas AM, Vila E. Middle cerebral artery alterations in a rat chronic hypoperfusion model. J Appl Physiol 112: 511–518, 2012. First published November 17, 2011; doi:10.1152/japplphysiol.00998.2011.—Chronic cerebral hypoperfusion (CHP) induces microvascular changes that could contribute to the progression of vascular cognitive impairment and dementia in the aging brain. This study aimed to analyze the effects of CHP on structural, mechanical, and myogenic properties of the middle cerebral artery (MCA) after bilateral common carotid artery occlusion (BCCAO) in adult male Wistar rats. Sham animals underwent a similar surgical procedure without carotid artery (CA) ligation. After 15 days of occlusion, MCA and CA were dissected and MCA structural, mechanical, and myogenic properties were assessed by pressure myography. Collagen I/III expression was determined by immunofluorescence in MCA and CA and by Western blot in CA. mRNA levels for 1A1, 1A2, and 3A1 collagen subunits were quantified by quantitative real-time PCR in CA. Matrix metalloproteinase (MMP-1, MMP-2, MMP-9, and MMP-13) and hypoxia-inducible factor-1α (HIF-1α) protein expression were determined in CA by Western blot. BCCAO diminished cross-sectional area, wall thickness, and wall-to-lumen ratio. Nevertheless, whereas wall stress was increased, stiffness was not modified and myogenic response was diminished. Hypoperfusion triggered HIF-1α expression. Collagen I/III protein expression diminished in MCA and CA after BCCAO, despite increased mRNA levels for 1A1 and 3A1 collagen subunits. Therefore, the reduced collagen expression might be due to proteolytic degradation, since the expression of MMP-1 and MMP-9 increased in the CA. These data suggest that BCCAO induces hypoxic remodeling by a mechanism that involves a reduction of collagen I/III in association with increased MMP-1 and MMP-9 and that decreases myogenic tone in major arteries supplying the brain.

bilateral common carotid artery occlusion; cerebral blood flow; hypoxia; vascular remodeling; myogenic response

BLOOD FLOW IRRIGATION to the brain is tightly regulated to ensure correct neuronal function. Nevertheless, several stroke risks factors can alter this homeostasis and lower cerebral blood flow (CBF) below a cerebral hypoperfusion threshold where cell function is compromised (14). Long-lasting reduction of CBF, known as chronic cerebral hypoperfusion, elicits multiple alterations, such as induction of oxidative stress (1, 37) and neuronal death (37), among others. In addition, experimental evidence suggests that cerebral hypoperfusion is associated with the decline of cognitive function and the development of several types of dementia (12, 16).

The middle cerebral artery (MCA) is one of the most extensive brain territories of irrigation (18), and reductions in MCA flow have been associated with cognitive disorders (19, 47). To better understand the effects of blood flow reduction in cerebrovascular disorders, a rat model of cerebral hypoperfusion has been well established by bilateral common carotid artery occlusion (BCCAO). Permanent BCCAO mimics some of the clinical conditions of cerebrovascular hypoperfusion associated with aging in humans (13, 16) and contributes to behavioral and cognitive deficits (48, 49, 55). In this animal model, the cerebrovascular flow drops sharply immediately after occlusion (7) and persists for several days after permanent BCCAO (36). However, compensatory redistribution via the vertebrobasilar network progressively recovers blood flow in almost all brain regions, and CBF values are indistinguishable from controls after 1 mo (7, 36).

Vascular remodeling involves structural changes in the vessel wall in response to hemodynamic stimuli. These alterations in composition/size of the vessel allow adaptation and repair (41). In resistance arteries, chronic increase or decrease in blood flow induces outward or inward remodeling, respectively (5, 15, 27, 40). The mechanisms involved in flow-induced remodeling have been mainly investigated in large and small arteries outside the brain. Nevertheless, previous studies have documented an enlargement and tortuosity of the basilar artery in response to permanent BCCAO (25, 44, 54). In addition, after 6 mo of BCCAO, the majority of main intracerebral arteries, including the MCA, increase their diameter, possibly contributing to the normalization of CBF (7). However, vascular remodeling is a complex process that usually takes place within hours to several days of the initiating stimulus (31). Thus a decrease of blood flow has been described to induce vascular remodeling in a period as short as 2 days (5). Moreover, changes in myogenic capacity have also been reported to modify vessel properties. For example, a decrease in MCA myogenic response is a hallmark of cerebral ischemia after transient MCA occlusion (9, 22, 23). In the present study, we aimed to analyze the effect of 15 days of BCCAO on structural, mechanical, and myogenic cerebrovascular properties and to determine potential mechanisms involved in these alterations.

MATERIALS AND METHODS

Animals. Thirteen- to 15-wk-old male Wistar rats (Charles River, Barcelona, Spain) were housed in a temperature-controlled room on a 12:12-h light-dark cycle and provided with access to food and water ad libitum. The investigation conforms to the “Guide for the Care and Use of Laboratory Animals” published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). Approval for this work was granted by the Universitat Autònoma de Barcelona Ethical Committee.

Bilateral common carotid artery occlusion. Rats were anesthetized by inhalation with 5% isoflurane in 0.5–1 l/min O2 and maintained with 2–2.5% isoflurane in 0.5–1 l/min O2. Adequacy of anesthesia
was confirmed by total absence of reflex responses (pinching the tail, pedal withdrawal, and pupillary reflex). Body temperature was monitored using a rectal probe and maintained at 37 ± 0.5°C during surgery. To produce analgesia, meloxicam (2 mg/kg) was administered subcutaneously 30 min before anesthesia and 24 and 48 h after surgery (1 mg/kg). Once the ventral neck was shaved and washed with 10% povidone iodine solution, BCCAO was performed as previously described (25, 52). Briefly, a ventral midline incision was made in the neck to expose both common carotid arteries (CA). Both CA were carefully separated from the vagus nerve, and occlusions were performed with a 5-0 silk suture (Suturas Aragó, Barcelona, Spain). The wound was closed, and the rats were allowed to recover from anesthesia and placed back into their cages. Sham animals underwent a similar surgical procedure without CA ligation. After 15 days, animals were killed by an overdose of sodium pentobarbital for tissue harvesting.

**Tissue preparation.** The entire brain and segments of CA upstream from the silk suture were removed and placed in ice-cold physiological salt solution (PSS) of the following composition (in mM): 112.0 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.1 KH₂PO₄, 1.2 MgSO₄, 25.0 NaHCO₃, and 11.1 glucose, maintained at 4°C and gassed with 95% O₂ and 5% CO₂. For pressure myography studies, the MCA was dissected out of the brain under a surgical microscope, cleaned from surrounding tissue, and kept in cold PSS. For immunofluorescence studies, CA and MCA were fixed with 4% phosphate-buffered paraformaldehyde (PFA; pH 7.4) for 45 min and washed three times with phosphate-buffered saline (PBS; pH 7.4). After clearing, vessels were placed in PBS containing 30% sucrose overnight, transferred to a cryomold containing Tissue Tek optimal cutting temperature (OCT) embedding medium, and frozen in liquid nitrogen. For Western blot and RT-PCR studies, the CAs were quickly frozen in liquid nitrogen. All samples frozen in liquid nitrogen were kept at −70°C until the day of the experiment.

**Pressure myography.** Structural, mechanical, and myogenic properties of the MCA were studied with a pressure myograph (Danish Myo Tech, model P100; J.P. Trading, Aarhus, Denmark), as previously described (22, 23). Briefly, vessels were placed on two glass microcannulas and carefully adjusted so that the vessel walls were parallel without stretching. Intraluminal pressure was then raised to 140 mmHg, and the artery was unbuckled by adjusting the cannulas. Afterwards, the artery was left to equilibrate for 1 h at 70 mmHg in gassed PSS (37°C). Intraluminal pressure was reduced to 10 mmHg, and a pressure-diameter curve (10–120 mmHg) was measured. Internal and external diameters (Dₑ and Dᵢₑ) were measured for 3 min at each intraluminal pressure. The MCA was then set to 70 mmHg and allowed to equilibrate for 30 min at 37°C in gassed calcium-free PSS (0 Ca²⁺; omitting calcium and adding 10 mM EGTA; Sigma-Aldrich, St. Louis, MO), and a second pressure-diameter curve was obtained in 0 Ca²⁺-PSS (passive conditions). Finally, the artery was fixed (70 mmHg) with 4% PFA for 45 min and prepared for immunofluorescence assays as indicated above.

Structural parameters such as wall thickness (WT), cross-sectional area (CSA), and wall-to-lumen ratio (W/L) were calculated as follows: WT = (Dₑᵢₑ − Dᵢᵢₑ)/2, CSA = (π/4)(Dᵢᵢₑ)² − (Dᵢᵢₑ)²), and W/L = (Dₑᵢₑ − Dᵢᵢₑ)/2Dᵢᵢₑ, where Dₑᵢₑ and Dᵢᵢₑ are the external and internal diameter observed for a given intravascular pressure in passive conditions (0 Ca²⁺-PSS), respectively. Mechanically parameters were calculated as described by Baumbach and Heistad (1989). Circumferential wall strain (ε) was calculated as (Dₑᵢₑ − Dᵢᵢₑ)/Dᵢᵢₑ, where Dᵢᵢₑ is the internal diameter at 10 mmHg, measured under fully relaxed conditions (0 Ca²⁺-PSS). Circumferential wall stress (σ) was calculated as (P × Dᵢᵢₑ)/2WT, where P is the intraluminal pressure (1 mmHg = 133.4 N/m²) and WT is wall thickness at each intraluminal pressure in 0 Ca²⁺ medium. Elastic modulus was calculated by fitting strain-stress data to σ = σ₀ exp (βε), where σ₀ is the stress at the original diameter (10 mmHg). The β value was used as an index of wall stiffness (33). Percentages of myogenic response were determined in each pressure using the following formula: 100 × Dᵢᵢₑ/Dᵢᵢₑ, where Dᵢᵢₑ and Dᵢᵢₑ are the internal and external diameters measured in active (2.5 mM Ca²⁺-PSS) and passive (0 Ca²⁺-PSS) conditions, respectively. Slopes of the myogenic response-pressure curves for individual vessels were determined by linear regression.

**Immunofluorescence.** Frozen transverse sections (14 μm) of MCA or CA were placed on gelatin-coated slides and air-dried (90 min). Sections were incubated with a rabbit primary polyclonal antibody against collagen III (1:30; Calbiochem, San Diego, CA). After being washed, rinses were incubated with the secondary donkey anti-rabbit IgG antibody conjugated to Cy3 (1:200; Jackson Immunoresearch Laboratories, West Grove, PA). Immunofluorescent signals were viewed by fluorescent laser confocal microscopy (Leica TCS SP2) using a ×20 (CA) or ×63 (MCA) objective. The Cy3-labeled antibody was visualized by excitation at 561 nm and detection at 600–700 nm. The specificity of the immunostaining was evaluated by omission of the primary antibody and processed as described above. Under these conditions, no staining was observed in the vessel wall in any experimental situation. Quantitative analysis of collagen III fluorescence was performed with MetaMorph 4.6 image analysis software (Universal Imaging, Molecular Devices, Downingtown, PA). The intensity of fluorescence per area was calculated in three rings of each animal, and the results were expressed in arbitrary units. All measurements were conducted blindly.

**Carotid artery morphometry.** Morphometric determination of the lumen, media, and vessel areas of the CA was performed using MetaMorph 4.6 image analysis software, as previously described (50). To determine the luminal area, the CSA enclosed by the internal elastic lamina was corrected to a circle by applying the form factor (π/4)r² to the measurement of the internal elastic lamina, where r is the length of the lamina. The vessel area was determined by the CSA enclosed by the external collagen, corrected to a circle by applying the same form factor (π/4)r² to the measurement of the external collagen. The CSA was calculated as follows: CSA = lₑ − lᵢ, where lₑ and lᵢ are the vessel and luminal area, respectively. Mean values were obtained from at least two different sections from the same artery.

**Western blot analysis.** Equal amounts of proteins (50 μg) from homogenized CA were loaded into individual lanes of minigels of 4–12% polyacrylamide gel and electrophoresed at 150 mA per well on Novex precast TBE gels; Invitrogen, Barcelona, Spain), transferred onto nitrocellulose membrane, and blocked in PBS-Tween 20 (0.1% vol/vol) containing 5% nonfat milk overnight at 4°C. Western immunoblotting was performed with monoclonal antibodies against MMP-1 (1:1,000; Calbiochem) and MMP-9 (1:1,000; Abcam, Cambridge, UK) or with polyclonal antibodies against collagen III (1:500; Calbiochem), MMP-13 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), MMP-2 (1:1,000; Abcam), and HIF-1α (1:1,000; Novus Biologicals, Littleton, CO). After being washed, membranes were incubated with peroxidase-conjugated secondary antibodies goat anti-mouse (1:2,000, for MMP-1 and MMP-9; Thermo Fisher Scientific, Barcelona, Spain), goat anti-rabbit (1:2,000, for collagen IIII, MMP-2, and HIF-1α; Thermo Fisher Scientific), and donkey anti-goat (1:2,000, for MMP-13; Santa Cruz Biotechnology). Immunocomplexes were detected using an enhanced horseradish peroxidase chemiluminescence system (SuperSignal West Pico chemiluminescence substrate; Thermo Fisher Scientific). The signal intensity of the bands was quantified using ImageJ software (National Institutes of Health, Bethesda, MD). All membranes were reblotted using monoclonal antibody anti-GAPDH (1:2,500; Santa Cruz Biotechnology) as a loading control. Data were normalized to corresponding values of GAPDH densitometry.

**Real-time quantitative RT-PCR.** Total RNA (tRNA) was isolated using Trizol (TRI Reagent) from frozen CA according to the manufacturer’s protocol. tRNA quality was evaluated by electrophoresis on a 1% denaturing agarose gel; tRNA concentration and purity were determined by measuring absorbance at 260 and 280 nm (A260/A280) by spectrophotometry (NanoDrop ND-1000; Thermo Fisher Scien-
tific). The samples with intact ribosomal RNA bands and with an A260/A280 ratio between 1.8 and 2.0 were used for cDNA synthesis. Total RNA (1 μg) was inversely transcribed to cDNA using a commercial kit (High-Capacity cDNA reverse transcription kit; Applied Biosystems, Foster City, CA) following the manufacturer’s conditions: first step of polymerase activation at 95°C for 10 min and 40 cycles composed of two steps, 10 min at 25°C (annealing step), followed by an extension step at 37°C for 2 h and a final step at 85°C for 5 min for heat inactivation. Gene expression for collagen subunits 1A1, 1A2, and 3A1 were quantified by quantitative real-time PCR (qRT-PCR) based on SYBR green fluorescence using the 18S ribosomal subunit of RNA (Hs99999901_s1) as an internal control with a GeneAmp 7500 PCR system (Applied Biosystems) following the manufacturer’s conditions: first step of polymerase activation at 95°C for 10 min and 40 cycles composed of two steps, denaturing at 95°C for 15 s and annealing/extension at 60°C for 1 min. The specific primer sequences for rodent were 1A1 (forward: 5′-AGAGTCTGAGTCCACCTAACAAC-3′ and reverse: 5′-CAGGCTGCGTCTTCGAGT-GAG-3′), 1A2 (forward: 5′-CTACTGTTGAAACCTGCATCCA-3′ and reverse: 5′-GGGCGCGGCTGATGGAG-3′), and 3A1 (forward: 5′-GGATCTGCTTCGAGATGAC-3′ and reverse: 5′-GCTGTTGCGGATATTCGACA-3′). Primers were designed from conserved regions of the studied genes: 1A1 (NM_007742.3), 1A2 (NM_007743.2), and 3A1 (NM_009930.2). qRT-PCR reactions were set following the manufacturer’s conditions. Threshold cycle (Ct) values obtained for each gene were referenced to r18S (ΔCt) and converted to the linear form using the term $2^{-\Delta\Delta Ct}$ as a value directly proportional to the copy number of cDNA and initial quantity of mRNA (35).

Statistical analysis. Results are means ± SE of the number (n) of rats indicated. The dependence of structural, mechanical, or myogenic properties on BCCAO was assessed by two-way analysis of variance (ANOVA) with repeated measures on the pressure factor. In the case of one single factor, unpaired Student’s t-test was used. Data analysis was carried out using GraphPad Prism 4 software. A value of $P < 0.05$ was considered significant.

RESULTS

Body weight. As shown in Table 1, body weight at day 0 was similar in both groups of rats. BCCAO induced a decrease ($P < 0.05$) of body weight on the first 2 days following surgery that recovered to the levels of sham animals by day 5. Afterwards, a similar progression in body weight increase was observed in both groups of rats.

HIF-1α. BCCAO caused sustained hypoxia in the vessels as assessed by the increased protein expression of HIF-1α in CA from BCCAO animals at day 15 (Fig. 1). HIF-1α is a transcription factor controlling signaling cascades that are involved in vascular remodeling (26).

Structural and mechanical properties of the MCA. Diameters of the MCA were measured over the pressure range (10–120 mmHg) under fully relaxed conditions (0 Ca²⁺-PSS), and the influence of BCCAO on MCA structural properties is shown in Fig. 2. After 15 days of BCCAO, vessel and lumen diameters of the MCA were not significantly modified (results not shown). However, CSA (Fig. 2A), WT (Fig. 2B), and W/L (Fig. 2C) were decreased ($P < 0.001$) in BCCAO compared with sham rats. Analysis of the passive mechanical properties showed that wall stress (Fig. 3A) was increased ($P < 0.05$) in MCA from BCCAO animals. Nevertheless, the stress-strain relationship (Fig. 3B) and the β values were similar (sham: 9.6 ± 1.2, n = 10; BCCAO: 9.4 ± 0.9, n = 9), demonstrating that wall stiffness remained unchanged.

Myogenic properties of the MCA. The extent of the constritor tone, also called myogenic response, is shown in Fig. 3C. In BCCAO vessels, myogenic response was decreased at all of the perfusion pressures tested, indicating a loss of constritor tone. Conversely, analysis of slopes of the myogenic response-pressure curves showed that this parameter was not altered in BCCAO (0.06 ± 0.02, n = 7) compared with sham vessels (0.08 ± 0.03, n = 10), indicating that myogenic reactivity is preserved after BCCAO.

Carotid artery morphometry. The morphometric analysis of rat CA demonstrated that CSA was decreased ($P < 0.01$) in BCCAO (1,716,612 ± 272,610 μm², n = 5) compared with sham vessels (3,245,512 ± 318,869 μm², n = 4). Expression of collagen I/III expression, shown as red fluorescence along the vessel wall of the MCA.
Permanent BCCAO in rats has been widely used as a model to study several cerebrovascular disorders (16, 51, 53). BCCAO causes CBF decreases immediately after occlusion and is an interesting approach to evaluate the effects of cerebral hypoperfusion (7). Our findings show that permanent BCCAO induces an increase of HIF-1α expression, an endogenous marker of hypoxia that is sustained for at least 15 days, evidencing a chronic exposure to factors inducing vascular remodeling (26). BCCAO decreased the MCA wall material, (Fig. 4A) and CA (Fig. 4B), was significantly reduced in both types of arteries from BCCAO rats compared with sham animals. The decrease of collagen I/III expression in MCA and CA after BCCAO was confirmed by Western blotting in CA (Fig. 5A). Analysis of mRNA levels for 1A1, 1A2, and 3A1 collagen subunits showed that all subunits were present in CA. Contrary to protein expression, BCCAO markedly enhanced mRNA expression of the 1A1 (Fig. 5B) and 3A1 (Fig. 5D) subunits (*P < 0.05), whereas results showed a decrease (***P < 0.001) in mRNA levels for the 1A2 subunit (Fig. 5C).

Protein expression of MMP. Expression of MMP-1 (Fig. 6A) and latent and active MMP-9 (Fig. 6B), analyzed by Western blotting, were increased (P < 0.05) in CA from BCCAO animals. In contrast, MMP-2 and MMP-13 expression remained unaltered (data not shown).

DISCUSSION

Permanent BCCAO in rats has been widely used as a model to study several cerebrovascular disorders (16, 51, 53). BCCAO causes CBF decreases immediately after occlusion and is an interesting approach to evaluate the effects of cerebral hypoperfusion (7). Our findings show that permanent BCCAO induces an increase of HIF-1α expression, an endogenous marker of hypoxia that is sustained for at least 15 days, evidencing a chronic exposure to factors inducing vascular remodeling (26). BCCAO decreased the MCA wall material,
and remodeling was paralleled by an increase in wall stress. Structural alterations of the MCA were accompanied by a decrease in collagen I/III protein expression by a mechanism that may involve increased degradation via MMP-1 and/or MMP-9. In addition, the myogenic response of the MCA was compromised. Therefore, we show alterations in the passive and active properties of an important cerebrovascular bed, which have not been, to our knowledge, previously evaluated after chronic cerebral hypoperfusion.

HIF-1 is a key transcription factor tightly regulated by cellular oxygen concentration and is composed of α- and β-subunits (45). It regulates oxygen-dependent expression of target genes involved in proliferation, erythropoiesis, and angiogenesis, among others (46). In the present study, we observed that after 15 days of permanent BCCAO there was an increase in CA HIF-1α protein expression, corroborating tissue hypoxia in this vascular bed.

Mounting evidence has established that structural variations in cerebral vasculature have an important role in brain function (10), and therefore, a better understanding of the mechanisms involved in structural changes into those arteries are of particular relevance and great clinical impact. In the present work, we focused on studying the effect of permanent BCCAO in MCA properties, because it is one of the longest intracranial arteries that irrigate an extensive territory in the brain, and decreases in MCA blood flow have been associated with...
cognitive deficits (19, 47). It is feasible that alterations in MCA properties may be involved in the cognitive decline described in the BCCAO-model (48).

Our study shows a reduction in CSA and WT of the MCA after 15 days of permanent BCCAO. Although we did not find a significant alteration in MCA diameter after 15 days of permanent BCCAO, our study is consistent with the notion that permanent BCCAO induced physiologically important vascular hypotrophic remodeling, since the decrease in the CSA was paralleled by a reduction in the W/L ratio of the MCA. The W/L ratio parameter allows for a better comparison of vessels, since it removes the effect of variability in vessel dimensions and is taken as an important predictor of cardiovascular events (32, 42). This observation is in agreement with previous studies in resistance arteries, where chronic decreases in blood flow induce hypotrophic remodeling (5, 40).

Wall stress has been postulated as an indicator of risk for brain injury (6, 8, 22). In the current study, the observed increase in wall stress correlates with a decrease in the CSA. It is well accepted that wall stress is the stimulus for subsequent growth (17), which is an adaptive response to preserve vessel integrity (3). We hypothesize that the increase of MCA wall stress observed after 15 days of permanent BCCAO occlusion might ultimately lead to MCA growth at later stages, likely mediated through the signaling cascades triggered by the expression of HIF-1α observed here. Consistently, increase in MCA diameter has been observed after 6 mo of permanent BCCAO (7).

Different mechanisms have been involved in the reduction of wall material. Among others, changes in extracellular matrix (ECM) components are well documented (4). ECM is a dynamic scaffold with the purpose of maintaining the integrity of blood vessels. We observed that after BCCAO, protein expression of collagen type I and III, the most abundant in the vascular wall (34), decreased in the MCA. Furthermore, CA (i.e., the arteries occluded) also experienced a similar pattern of decrease in CSA and collagen I/III protein expression. The parallelisms of MCA and CA results summed to the very limited amount of sample we could obtain per vessel/animal drove us to perform the next sets of experiments for protein/mRNA expression in CA only. Results from quantitative PCR analysis for the expression of each subunit of collagen I and III in the vessel wall revealed an overall opposite effect compared with protein expression. Whereas both immunofluorescence and Western blot analysis showed a decrease of collagen I/III protein expression in animals with BCCAO, mRNA expression for collagen 1A1 and 3A1 were upregulated in those animals. Although mRNA expression of the 1A2 collagen subunit was downregulated following BCCAO, the low expression of this subtype (about 10,000 less than other subtypes) should not account for the decrease in collagen protein expression observed in this study. More likely, changes in posttranslational mechanisms or collagen degradation would contribute to these effects.

MMPs are endopeptidases important for the regulation of ECM dynamics and have been involved in ECM degradation. MMPs in the vessel wall are regulated by multiple stimuli, including hemodynamic forces such as low blood flow. We found that the decreases in collagen I/III deposition in MCA and CA were accompanied by an increase in MMP-1 and -9 (including active forms) expression in CA. These findings agree with the remodeling induced by blood flow reduction, where MMP activation has been observed (2, 24). Therefore, CBF attenuation induces collagenolytic activity by MMPs that will contribute to the MCA hypotrophy observed at this stage. The resulting remodeling elevates wall stress, which in turn will signal for the further development of hypertrophy in an attempt to decrease wall tension. However, low-flow-induced remodeling independent of MMPs has also been described (15).

Alterations in vascular mechanical properties may take part in the cerebral disturbance caused by ischemic processes (23). Arterial stiffness is influenced by ECM proteins, and, in general, collagen contributes to enhanced stiffness (4). MCA from BCCAO rats showed diminished collagen I/III expression, and thus decreased stiffness would be expected. Instead, here wall stiffness remained unchanged after 15 days of permanent BCCAO. Similarly, paradoxical alterations of collagen and compliance have already been reported under other conditions (20, 43). Previous studies support that, besides collagen content, collagen organization contributes to define the stiffness of the arterial wall (4, 21). Furthermore, not only collagen but...
also other ECM components (i.e., elastin) are involved in arterial stiffness and might counteract the possible effects of decreased collagen. Finally, we cannot exclude that collagen reduction was an early event preceding further alterations in stiffness at later time points.

Numerous studies have only attempted to analyze the influence of vascular remodeling in several disease states. However, CBF is tightly regulated by myogenic mechanisms to maintain a relatively stable cerebral perfusion. Myogenic response as a function of pressure reveals the extent of the constrictor tone, whereas myogenic reactivity refers to vasoconstriction of the vessel to pressure once tone is present (10). In the present study, although myogenic reactivity was preserved, myogenic response was decreased at all of the perfusion pressures tested. It is well accepted that after a transient ischemic episode, one of the mechanisms involved in myogenic impairment of rat cerebral arteries is the increased peroxynitrite production causing loss of F-actin (22, 28, 29). However, vascular myogenic properties are also intricately linked to changes in wall structure. The vascular ECM is subject to tensile force produced by blood pressure and transferred through integrins. Among others, these integrins can form signaling complexes that regulate cytoskeletal dynamics, indirectly regulating myogenic capacity (30). ECM disruption (i.e., collagen degradation by MMP) might directly impact these signaling complexes, contributing to myogenic impairment. However, the relationship between arterial remodeling and myogenic autoregulation is still far from being well understood.

In a previous study, we showed a decrease in MCA myogenic response from normotensive compared with hypertensive rats after transient MCA occlusion/reperfusion, contributing to an explanation of the smaller infarct volume in the former (23). Furthermore, we subsequently observed that reduction of the cerebral infarct size by an antioxidant treatment was not coupled to normalization of the MCA myogenic response (22). In those studies, including the present one, decreased myogenic response was accompanied by preserved myogenic reactivity. Together, these findings suggest that decreased MCA myogenic response could be considered as an adaptive response to facilitate and/or preserve blood flow irrigation, thus limiting tissue damage.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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