Identification of a region of rat chromosome 1 that impairs the myogenic response and autoregulation of cerebral blood flow in fawn-hooded hypertensive rats


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The fawn-hooded hypertensive (FHH) rat is a genetic model of hypertension (23) that is very susceptible to renal end-organ damage (11). We reported that the myogenic response of renal interlobular arteries and AR of renal blood flow (RBF) is impaired in FHH rats compared with fawn-hooded low-pressure rats (29, 30). More recently, we found that the substitution of a 99.4-Mbp region of rat chromosome 1 (RNO1) from Brown Norway (BN) to FHH rats between markers D1Rat76 and D1Nor107, along with a 12.9-Mb region in the q-terminus of RNO1, restored AR of RBF and reduced proteinuria in a FHH.1BN dual congenic strain (31). Subsequently, we developed a congenic strain that narrowed the region of interest to a smaller 4.7-Mb region on RNO1 (strain B, Fig. 1). However, no studies have examined whether the impaired myogenic response in FHH rats is specific to the renal circulation or reflects a more general vascular defect. Indeed, a recent report suggested that the myogenic response of mesenteric arteries is not altered in FHH rats (20). Thus the present study examined the hypothesis that FHH rats have a mutation in a gene in the previously described region on RNO1 (31) that impairs the myogenic response in cerebral arteries and AR of CBF. This hypothesis was tested by comparing AR of CBF, the myogenic response of isolated middle cerebral arteries (MCAs) and cerebral ischemia-reperfusion (IR) injury in FHH rats and in FHH.1BN congenic strains in which a 2.4-Mbp region of RNO1 from 258.8 to 321.7 Mb was transferred from BN rats into the FHH rat genetic background.

METHODS

General. Experiments were performed on 118 9–12-wk-old male FHH and FHH.1BN congenic rats that were obtained from inbred colonies maintained at the University of Mississippi Medical Center (UMMC) and Medical College of Wisconsin (MCW). All the rats were housed in the Animal Care Facilities at the UMMC and MCW, which are both approved by the American Association for the Accreditation of Laboratory Animal Care. The rats had free access to food and water throughout the study. All protocols received approval for the use of animals in these studies.
by the Animal Care Committees of both institutions. The breeding colonies were maintained on a rodent diet purchased from LabDiet (PMI Nutrition International, Brentwood, MO) containing 0.28% NaCl. After being weaned, the pups were switched to a purified AIN-76 rodent diet containing 0.4% NaCl (Dyets, Bethlehem, PA). Protocol 1: AR of CBF in FHH and FHH.1BN congenic strains. These experiments were performed on 9–12-wk-old male FHH rats and FHH.1BN congenic strains (Fig. 1, strains A–E). The rats were anesthetized with ketamine (30 mg/kg im, Phoenix Pharmaceutical, St. Joseph, MO) and Inactin (50 mg/kg ip, Sigma, St. Louis, MO) and placed in the supine position. A polyethylene cannula (PE-240, Intramedic Fisher Scientific) was placed in the femoral artery for the measurement of arterial pressure and femoral vein for an intravenous infusion of 0.9% NaCl solution at a rate of 100 µl/min to replace surgical fluid losses. The rats were placed on a heating pad to maintain temperature at 37°C.

After the head of the rat was secured in a stereotaxic apparatus (Stoelting, Wood Dale, IL), the scalp was removed over the parietal cranial bone. A 3 × 3 mm area of the left and right parietal bones, 2 mm distal to bregma and 7 to 8 mm from center line, was thinned with a handheld low-speed drill until superficial pial vessels became visible. The windows were made slowly and often cooled with saline. CBF was monitored bilaterally with a laser-Doppler flow meter (Perimed, PF5001, Jarfalla, Sweden) and 1-mm flow probes. The probes were lowered into position using micromanipulators, and a drop of mineral oil was applied to the probe tip to provide optical coupling. After surgery and a 30-min equilibration period, MAP was adjusted to 90–100 mmHg by deepening the depth of anesthesia using a low dose of pentobarbital sodium (1–5 mg/kg iv). Baseline regional CBF (rCBF) was measured at this level of MAP, and pressure was then elevated in steps of 10–15 mmHg up to 150 mmHg by graded intravenous infusion of phenylephrine (0.5–5 µg/kg/min) via the femoral vein. MAP was maintained for 3–5 min until a new steady-state level of rCBF was obtained. After the relationship between rCBF and elevations in MAP was measured, blood pressure was allowed to return to control by withdrawing phenylephrine infusion. MAP was then sequentially lowered in steps of 10 to 40 mmHg by the withdrawing of small amounts of venous blood. MAP was allowed to stabilize for 5 min at each level of MAP to ensure buildup of metabolic mediators, after which rCBF was recorded. rCBF was expressed as a percentage of baseline laser-Doppler flow signal. The CBF autoregulatory index (AI) was calculated as the percent change in CBF-percent change in MAP. According to this analysis, an index

Fig. 1. Genetic map of the introgressed region in FHH.1BN congenic strains. Genomic segments from the fawn-hooded hypertensive (FHH) and Brown Norway (BN) rat are presented as white and black bars, respectively. Top: formal designations of congenic strains A–E based on the recommended “Guidelines for Nomenclature of Mouse and Rat Strains” presented on the Rat Genome Database (RGD@ncw.edu). Left: some of the polymorphic genetic markers used to genotype these strains. Right: 16 genes that map to the 2.4-Mbp region of interest on rat chromosome 1 according to the Rat Genome Database (RGD, RGSC Genome Assembly v3.4).

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of 0 indicates perfect AR of CBF, whereas an index of 1 is characteristic of a circulation with a fixed vascular resistance.

**Protocol 2: myogenic response in isolated MCAs.** The rats were anesthetized with 4% isoflurane, and the brain was removed and placed in ice-cold physiological salt solution containing (in mM) 119 NaCl, 4.7 KCl, 1.17 MgSO4, 1.6 CaCl2, 1.2 NaHCO3, 1.18 NaH2PO4, 0.03 EDTA, and 10 glucose (pH 7.4). MCAs were removed and mounted on glass pipettes and pressurized to 40 mmHg. The cannulated vessels were visualized using a videomicroscopy system (model KP130; Hitachi, Denshi, Tokyo, Japan), and a stereomicroscope (model DRC; Zeiss, Oberkochen, Germany). Vascular diameter was measured using a videomicrometer (VIA-100, Boeckeler Instruments, Tucson, AZ). After a 30-min equilibration period, a control myogenic response curve was constructed by measuring internal diameter of the vessels in response to increases in transmural pressure from 40 to 140 mmHg in steps of 20 mmHg. After a control relationship was determined, the bath was replaced with Ca2+–free physiological salt solution and the measurements repeated.

**Protocol 3: transient MCA occlusion.** Anesthesia was induced with 4% isoflurane and maintained with 1.5% isoflurane using a gas mixture of 30% oxygen and 70% nitrogen through an inhalation mask. Body temperature was continuously monitored and maintained at 37°C. Catheters were placed in the femoral artery and vein for the measurement of MAP and the infusion of drugs. The skull was exposed, and the bone was thinned bilaterally, 6 mm lateral and 2 mm posterior to bregma, for measurement of rCBF using laser-Doppler flowmetry. Focal cerebral ischemia was induced using the intraluminal suture method as previously described (19, 21). Briefly, the right common, internal and external carotid arteries were exposed through a midline incision of the neck. An 18-mm segment of nylon suture (4-0; Ethicon) coated at the tip with silicon (Xantopren VL plus; Heraeus Kulzer Dental Products Division, South Bend, IN) was introduced from the bifurcation into right internal carotid artery after ligation of the right common and external carotid arteries. The suture was advanced to the origin of the MCA and the internal carotid artery until rCBF fell by 80%. After 60 min of MCA occlusion (MCAO), the suture was removed to allow reperfusion. rCBF and MAP were continuously recorded for a 60 min ischemic period, followed by 120 min of reperfusion. The femoral catheters were removed, and the animal was allowed to recover from anesthesia. After 24 h of reperfusion, the rats were reanesthetized with 4% isoflurane and euthanized by decapitation. The brains were removed, cut into six 2-mm-thick coronal sections, and stained with 2% 2,3,5-triphenyltetrazolium chloride at 37°C for 30 min to measure infarct volume. After being stained, the slices were fixed with 10% buffered formalin solution. Cortical and subcortical infarct volumes were measured using MetaMorph digital imaging software. Infarct volume was expressed as a percentage of the area of the ischemic hemisphere.

**Statistical analysis.** Data are presented as mean values ± SE. The significance of differences in mean values was determined using a paired t-test (2 samples) or an analysis of variance for repeated measures, followed by a Holm-Sidak test. A P < 0.05 was considered to be significant.

**RESULTS**

**Protocol 1: AR of CBF in FHH and FHH.1BN congenic strains.** A genetic map comparing the regions of BN RNO1 introgressed into the various FHH.1BN congenic strains are presented in Fig. 1. We previously reported that transfer of a 99-Mbp region of rat RNO1 from BN rats from markers D1Rat183 to D1Rat76 along with a 4.7-Mbp region on q-terminal of RNO1 restored AR of RBF in a FHH.1BN dual congenic strain B (31). However, it remains to be determined whether the genes in the region specifically alter the myogenic response in the kidney or alter vascular function in general.

Thus, in the present study, we compared AR of CBF and the myogenic response in cerebral arteries in FHH rats and this dual congenic strain B. We also studied AR of CBF in a FHH.1BN AR− control strain A that is identical to strain B in the 99-Mbp region of RNO1 but excludes the 4.7-Mbp region of interest between genetic markers D1Rat376 and D1Rat225. In addition, we created and studied two additional dual congenic strains C and D that split the 4.7-Mbp region of interest. FHH.1BN congenic strain C has a 2.3-Mbp region of BN RNO1 introgressed between genetic markers D1Rat376 and D1Rat09, whereas strain D has a 2.4-Mbp region of BN RNO1 introgressed between markers D1Rat09 and D1Rat225 in a genetic background identical to the dual FHH.1BN congenic strain B. Afterward, we found that AR of CBF was restored in the dual congenic strain D and we backcrossed them with FHH rats and intercrossed the progeny to create a new minimal FHH.1BN congenic strain E in which the 99-Mbp region of BN RNO1 in congenic strains A through D reverted back to FHH alleles, whereas the 2.4-Mbp region of BN RNO1, spanning markers D1Rat09 to D1Rat225, was retained. The rat genome database (http://rgd.mcw.edu) currently reports that there are

![Fig. 2. Autoregulation of CBF in 12-wk-old FHH rats and FHH.1BN congenic strains. A: relationship between CBF measured by laser-Doppler flowmetry and mean arterial pressure (MAP). B: autoregulatory indexes (AIs) calculated for an elevation in MAP from 100 to 150 mmHg. Mean values ± SE are presented. Numbers in parentheses indicate number of animals studied per strain. *Significant difference from the control value at 100 mmHg within a strain; †significant difference from the corresponding value in FHH rats.](http://alphapub.org/doi/10.1152/ajpheart.00622.2012)
16 known and predicted genes in this region (Fig. 1). Of these genes, only 3 [adducin-γ (Add3), dual-specificity phosphatase 5 (DUSP5), and X-prolylaminopeptidase 1] have any known influence on cardiovascular function.

A comparison of AR of CBF in FHH and the FHH.1BN congenic strains A–E are presented in Fig. 2. CBF increased by 40% in response to an elevation in MAP from 100 to 150 mmHg in FHH rats. Similar results were obtained in AR − FHH.1BN congenic strains A and C in which regions of BN RNO1 other than the 2.4-Mbp region of interest between markers D1Rat09 and D1Rat225 were introgressed. AR of CBF was markedly improved in the AR + dual congenic strains B and D in which regions spanning the 2.4-Mbp region of BN RNO1 was introgressed. Similarly, AR of CBF was also improved in the minimal FHH.1BN congenic strain E. A summary of the AIs observed in FHH rats and the congenic strains are presented in Fig. 2B. The CBF AIs averaged ~0.8 in FHH rats and AR − congenic strains A and C. The CBF AIs were significantly lower in AR + FHH.1BN congenic strains B, D, and E.

A comparison of the CBF autoregulatory responses of FHH rats and the congenic strains to reductions in MAP produced by graded hemorrhage are presented in Fig. 3. CBF decreased by about 40% as MAP was reduced by 50% from 80 to 40 mmHg. The CBF AIs averaged 0.8 in FHH rats and AR − congenic strains A and C. The CBF AIs were significantly lower in AR + FHH.1BN congenic strains B, D, and E.

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A comparison of the myogenic response of MCAs isolated from FHH rats and FHH.1BN congenic strains is presented in Fig. 4A. The myogenic response of MCAs isolated from FHH rats was impaired, and the diameter of these vessels increased significantly from 140 ± 14 to 157 ± 18 μm when transmural pressure was increased from 40 to 140 mmHg. The MCAs isolated from the AR − FHH.1BN congenic strain A also exhibited a minimal myogenic response, and diameter only fell from 144 ± 17 to 129 ± 23 μm in response to the same elevation in transmural pressure. In contrast, the diameter of MCAs isolated from FHH.1BN congenic strains B, D, and E decreased by 35, 40, and 35%, respectively, when transmural pressure was elevated from 40 to 140 mmHg (Fig. 4A). We did not study the myogenic response in MCAs obtained from line C because this region was excluded from consideration as they failed to exhibit AR of CBF. After calcium was removed from the bath, the pressure diameter relationship of MCAs isolated from FHH rats was unchanged, indicating that these vessels fail to develop any myogenic tone. In contrast, the diameter of vessels isolated from congenic strains B, D, and E increased, and these vessels dilated in response to elevations in transmural pressure. However, to simplify the figure, only the data from FHH rats and representative data from line C are presented. Numbers in parentheses indicate number of vessels studied per strain. Significant difference from the corresponding value in FHH rats.
I/R injury, so infarct size and edema volume could not be studied in this strain. Poor survival of these rats has been associated with bleeding disorder that is due to a shared mutation in the RAB38 gene located in the 99-Mbp region of RNO1 that is substituted in the dual congenic strains A, B, and D. Similarly, infarct sizes were not studied in congenic strain C for the reasons discussed earlier. Infarct size and edema volume averaged 39 ± 3 and 12 ± 2%, respectively, in AR− FHH.1BN congenic strain A that like FHH rats does not autoregulate CBF. Both of these values were significantly increased relative to the comparable values observed in FHH AR+ congenic strains B and D in which AR of CBF is restored (Fig. 6, A and B).

A comparison of infarct sizes and edema volumes in FHH.1BN congenic strains A, B, and D is presented in Fig. 6. FHH rats and strain E did not survive for 24 h following I/R injury, so infarct size and edema volume could not be measured.

**DISCUSSION**

The present study compared AR of CBF and myogenic response of MCAs in FHH rats and FHH.1BN congenic strains that included or excluded a 2.4-Mbp region of BN rat RNO1 introgressed into FHH rat genetic background. The new findings of the present study are that AR of CBF to elevations in MAP is markedly impaired in FHH rats, and this was associated with a lack of a myogenic response in isolated MCAs. MCAs from FHH rats failed to develop any myogenic tone, as the pressure-diameter relationship was not significantly different in vessels studied in the presence and absence of extracellular calcium. We found that transfer of a the same 4.7-Mbp region of BN RNO1 between markers D1Rat376 and D1Rat225 in the FHH.1BN dual congenic strain B that was previously reported to restore AR of RBF also improved AR of CBF. The myogenic response of the MCA isolated from this strain was also restored. This indicates that the defect in myogenic tone is not unique to the renal circulation but is related to a more generalized defect in vascular function. Subsequent studies using two newly created FHH.1BN congenic strains C and D narrowed the region of interest to a 2.4-Mbp region of RNO1 spanning genetic markers D1Rat09 and D1Rat225. Finally, to exclude the possibility that the restoration of the myogenic response and AR of CBF in the dual congenic strains B and D is due to an interaction of BN genes in the two introgressed regions, we created and studied a minimal FHH.1BN congenic strain E. The results obtained using this strain indicate that there is a gene in the 2.4-Mbp region of RNO1 containing 16 known and predicted genes that can restore the myogenic response in cerebral arteries and AR of CBF.
Since FHH rats are reported to be a hypertensive strain, one might consider that the differences in the myogenic response of the MCAs of FHH rats and the congenic strain might be secondary to differences in blood pressure between the strains. However, the term fawn-hooded hypertensive strain is a bit of a misnomer since we have reported that they only exhibit a small 10-mmHg increase in blood pressure relative to other strains between 18 and 21 wk of age (14, 31). Blood pressure measured by telemetry was not significantly different in 9–12-wk-old FHH rats and congenic strains A and B (31). More recently, we have also compared blood pressure measured by telemetry in FHH rats and all of the congenic strains used in the present study. We found that blood pressure was not significantly different in any of the strains between 9 and 15 wk of age and averaged ~120 mmHg (R. J. Roman and M. Burke, unpublished observations). Thus it is unlikely that the phenotypic changes we have observed in the myogenic response of cerebral arteries and AR of CBF is secondary to structural or adaptive changes in the cerebral vasculature.

The present study also examined the CBF responses in FHH and the congenic strains to reductions in MAP caused by graded hemorrhage. The results in the FHH rats and congenic strain A were very consistent with the lack of AR to elevations in pressure in these strains and indicated that there was a ~40% fall in CBF in response to a 50% reduction in MAP. This suggests that FHH rats have very little ability to compensate to reductions in MAP by dilating the cerebral circulation. In contrast, CBF fell significantly less in the congenic strains B, D, and E in which the myogenic response was restored.

These findings suggest that there is likely some degree of resting myogenic tone in the cerebral circulation in these congenic strains that could be withdrawn in response to a fall in MAP from 80 to 40 mmHg. The lack of compensation to reductions in MAP seen in the FHH and the FHH.1BN congenic strain A was a bit surprising since the release of metabolic vasodilators such as acetylcholine, ATP, adenosine, epoxygenic acids, and nitric oxide are thought to contribute to this response. The lack of response implies that whatever the genetic defect that impairs the myogenic response in cerebral arteries, it must also affect the ability of these vessels to dilate to these metabolic mediators as well.

One would also predict that the lack of AR in the brain of FHH rats should make them more vulnerable to the effects of I/R injury. The restoration of myogenic response and AR of CBF in the AR+ FHH.1BN congenic strains suggests that they should be more able to prevent the transmission of elevated systemic pressure to the brain following elevations in MAP and opposes the development of cerebral edema and injury. In support of this later conclusion, we found that FHH rats and the AR− FHH.1BN congenic strain A exhibited a exaggerated and prolonged hyperemia following t-MCAO relative to FHH.1BN congenic strains B and D in which AR was restored. Moreover, infarct size and edema of the brain was significantly greater in the congenic strain that did not autoregulate CBF than in those in which AR was restored. These results are consistent with the view that activation of the myogenic response contributes to the rapid normalization of CBF following cerebral I/R. This limits the rise in capillary pressure and swelling of the brain which then influences cerebrospinal fluid pressure and ultimately blood flow and infarct volume in the ischemic penumbra.

The myogenic response is an intrinsic property of the vascular smooth muscle cell that involves calcium influx via voltage-gated calcium channels that leads to vasoconstriction (3). Increase in intracellular Ca2+ activates large-conductance K+ channels that hyperpolarize the membrane and oppose the myogenic response (8). The genes in the region of interest on RNO1 between D1Rat09 and D1Rat225, which impair myogenic response in FHH rats, remains to be determined. The region of interest on RNO1 in minimal congenic FHH.1BN strain E in which CBF AR was fully restored contains only 16 genes. Examination of the genes in the region indicates that there are two genes, DUSP5 and Add3, that may have some impact on vascular function. DUSP5 is a member of the DUSP gene family that dephosphorylates critical signaling molecules such as MAPK, ERK, and JNK that are involved in pressure- or stretch-induced myogenic responses (15, 18, 25). The other gene of interest is the Add3 gene, which was one of the first genes reported to cosegregate with the development of hypertension in a cross of Milan normotensive and Milan hypertensive rats (32). Subsequently, evidence has emerged linking mutations in adducin isoforms to the development of hypertension and other forms of cardiovascular disease in rats and humans (1, 5, 13, 26–28). Add3 promotes the spectrin-actin binding and controls the rate of actin polymerization by capping actin filaments at the plasma membrane (16, 17). Add3 is also a calmodulin binding protein and serves as a substrate for protein kinase C and Rho kinase (9, 10), both of which are important regulators of vascular tone. Furthermore, the administration of a pseudosubstrate inhibitor for protein kinase C has been shown to prevent the myogenic response in cerebral arterioles (12).

Perspectives. The results of the present study suggest that the lack of AR of CBF in FHH rats is due to impaired myogenic response in cerebral arteries, and there is a gene(s) that lies in the 2.4-Mbp region of RNO1 transferred from BN rats that restores the AR of CBF in FHH.1BN minimal congenic strain. The impaired myogenic response may enhance the transmission of systemic pressure to the cerebral microcirculation that promotes cerebral I/R injury in FHH rats. These results may provide information critical to the identification of new genes and therapeutic targets for the treatment of neurological damage associated with impaired AR CBF in hypertensive and diabetic patients and following stroke or traumatic brain injury and/or the cognitive decline associated with vascular dysfunction in aging.

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AUTHOR CONTRIBUTIONS

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


