SOD1 overexpression prevents acute hyperglycemia-induced cerebral myogenic dysfunction: relevance to contralateral hemisphere and stroke outcomes

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More than one-third of acute ischemic stroke patients have acute hyperglycemia upon admission (1, 42), which leads to poor clinical outcomes and a higher risk of mortality (1, 7, 32, 43). Intriguingly, patients who have hyperglycemia but no history of diabetes suffer the poorest outcomes (24). We previously showed that a modest acute elevation in blood glucose at the time of stroke amplifies vascular injury and neurological deficits (15). In addition, previous studies have reported a reduction in regional cerebral blood flow (CBF) during acute hyperglycemia (14, 21), which could accentuate brain dysfunction after ischemia (13, 22). Together, all these findings emphasize the detrimental impact of acute hyperglycemia on the cerebrovasculature and the importance of an intact vascular system in functional outcomes after stroke.

Myogenic reactivity is an intrinsic property of smooth muscle cells to constrict in response to pressure by which the brain can maintain adequate blood flow during changes in perfusion pressure (12). We recently showed that ischemia-reperfusion (I/R) injury has a short-term global effect, impairing cerebrovascular myogenic reactivity and lowering perfusion in both ischemic and contralateral hemispheres (11). However, the role of contralateral myogenic dysfunction on stroke outcomes and especially under conditions that amplify I/R injury remain unknown. Thus, our first goal in the present study was to test the hypothesis that hyperglycemia worsens contralateral myogenic dysfunction and that enhancement of contralateral myogenic tone improves stroke outcomes.

It is well established that increased generation of ROS could alter myogenic function after I/R with or without hyperglycemia (11, 35, 38). We have previously shown that I/R injury has a short-term global effect impairing myogenic reactivity in both hemispheres, 2) impaired myogenic tone was due to excess peroxynitrite generation and actin nitration leading to actin depolymerization, and 3) middle cerebral arteries (MCAs) isolated from diabetic rats exposed to oxygen-glucose deprivation ex vivo experience loss of tone due to excess peroxynitrite generation and nitration (11, 26). Therefore, antioxidant agents are a promising therapeutic intervention for acute hyperglycemic stroke. Cu/ZnSOD (SOD1) is one of the antioxidant agents that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Previous studies using SOD1 transgenic rats have supported the beneficial role of SOD1 in improving stroke outcomes after I/R injury (25, 30). Therefore, the second goal of the present study was to test the hypothesis that improvement of myogenic dysfunction by local SOD1 overexpression limits stroke injury and improves functional outcomes.

Materials and Methods

Animals. Experiments were performed on weight-matched (250–350 g) male Wistar rats, SOD1 transgenic rats, and Sprague-Dawley (SD) rats (Harlan, Indianapolis, IN), which served as the control group for experiments involving transgenic animals. Animals were subjected to MCA occlusion (MCAO) with and without acute hyperglycemia.

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Animals were housed at the Georgia Regents University animal care facility, which has been approved by the American Association for Accreditation of Laboratory Animal Care. All protocols were approved by the Institutional Animal Care and Use Committee. Animals were fed standard rat chow and tap water ad libitum. All animals were euthanized by decapitation after being anesthetized with pentobarbital sodium (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI).

**Model of ischemia.** Focal cerebral ischemia was achieved in a blinded manner for the various groups using the monofilament suture MCAO model previously described by our group (15). The skin on the cervical region was incised to access the common carotid artery. The external carotid artery was separated, ligated, and severed. Nylon suture with a rounded tip was inserted into the internal carotid artery to approach the origin of the MCA. The nylon suture occluding the MCA was secured along the external carotid artery at its base, and the incision was closed. Rats were subjected to sham operation (sham) or 30 min of MCAO followed by 45 min or 24 h of reperfusion. Acute hyperglycemia was achieved by 2 ml ip of 40% glucose injection 10 min before MCAO and was maintained during 24 h of reperfusion by another injection at the end of the MCAO. Blood glucose levels were measured from a tail vein using a glucometer (Freestyle, Alameda, CA) and reported at baseline, MCAO, and reperfusion. In sham groups, animals were dissected in the neck region, and the common carotid artery was ligated as in stroke surgery but was not subjected to MCAO. At the end of 30 min, the suture was released, and animals were euthanized similarly to the MCAO group.

**CBF measurement.** Cerebral perfusion was measured by a scanning laser Doppler imaging system (PeriScan PIM 3 System). In brief, the top of the skull was exposed by a median incision of the skin, which is supplied by the MCA. The laser beam was directed at the skull surface (2 mm posterior and 5 mm lateral to the bregma) by a moving-mirror system in the scanner without tissue contact. In this system, a built-in photo detector identifies the reflected light from moving blood cells within 0.5 cm of the cortical surface, and a color-coded image is acquired based on the concentration and mean velocity of these blood cells using LDPIwin software (Perimed, North Royalton, OH).

**SOD1 transgenic rats.** Heterozygous SOD1 transgenic rats on the SD background, with increased SOD1 activity, were generously provided by Dr. Pak H. Chan. SOD1 transgenic rats were genotyped using PCR by a mixture of primer sequences (5'-CCATCTC-CTTTTGTGAGACA-3' and 5'-AGGCATGGAGTCAATGGGAG-3', IDT, San Diego, CA), which yielded a 505-bp band.

**Stereoactive injections.** Wistar rats were anesthetized with isoflurane and immobilized on a stereotaxic device 2–3 wk before MCAO. All protoclos were approved by the Institutional Animal Care and Use Committee. Animals were fed standard rat chow and tap water ad libitum. All animals were euthanized by decapitation after being anesthetized with pentobarbital sodium (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI).

**Pressurized arteriograph system.** MCA segments from ischemic and contralateral hemispheres were quickly excised and pressurized in an arteriograph chamber (Living Systems, Burlington, VT) at 15 mmHg for 1 h within 45 min of isolation to ensure vessel viability (11). Pressure-diameter curves were obtained first in the presence of Ca²⁺ (active condition) and then in Ca²⁺-free buffer (passive condition) with the addition of 0.2 mM papaverine hydrochloride. A video dimension analyzer connected to the arteriograph system was used to measure wall thickness and lumen diameter at pressures ranging from 0 to 180 mmHg in 20-mmHg increments. Using the wall thickness and lumen diameter measurements, percent myogenic tone [percent myogenic tone = 1 – (active outer diameter/passive outer diameter) × 100] was determined.

**Neurovascular injury assessment.** All animals were anesthetized with pentobarbital sodium (Fatal-Plus, Vortech Pharmaceuticals) and underwent intracardiac perfusion of ice-cold saline to flush blood out of the vessels at the end of 24 h of reperfusion. Brains were extracted, sliced into 2-mm slices, and stained with 2% solution of 2,3,5-triphenyltetrazolium chloride (Sigma) to evaluate tissue viability and delineate the infarcted area. Images were captured using a digital scanner, and SPOT Advanced 3.4 software (Diagnostic Instruments, Sterling Heights, MI) was used to quantify grossly visible infarction zones. The infarct volume was determined as a percentage of the ischemic hemisphere. Edema is reported as the percent increase in ischemic hemisphere size to the contralateral hemisphere.

**Neurological outcomes assessment.** Beam walk and grip strength tests, which assess sensorimotor function, were performed at baseline and at the end of 24 h of reperfusion. Forelimb grip strength was determined using a digital grip strength meter (Columbus Instruments, Columbus, OH) (46). Beam walk evaluation was done based on the seven-point scale method previously described by Feeney et al. (18).

**Statistics.** The area under the curve (AUC) was calculated across intraluminal pressure for vessels from each animal for myogenic tone (40 to 180 mmHg) using NCSS 2007 (NCSS, Kaysville, UT) and was used in the analyses for these variables. Myogenic tone AUC was analyzed using two stroke (sham vs. ischemia or nonischemia) by two hyperglycemia (no vs. yes) ANOVA with interactions where the ischemic and nonischemic sides of the brain were analyzed separately. Data from Wistar rats were analyzed using two stroke (sham vs. MCAO) by two hyperglycemia (no vs. yes) ANOVA to determine the effect of stroke and hyperglycemia on blood glucose at baseline, MCAO, and reperfusion. One-way ANOVA (sham, MCAO, MCAO + hyperglycemia) was used to determine the effect of MCAO and hyperglycemia on myogenic tone AUC for SD and SOD1 transgenic rats. A two-sample t-test was used to establish differences in SOD activity and expression (SD vs. SOD1). A series of two SOD1 (no vs. yes) by two hyperglycemia (no vs. yes) ANOVAs with interactions were used to determine the effect of hyperglycemia and SOD1 on blood glucose at baseline, MCAO, and reperfusion as well as infarct size, edema, beam walk, grip strength, and nitrotyrosine levels. Two stroke (sham vs. MCAO) by two SOD1 (no vs. yes) ANOVA was used to determine the effect of MCAO and SOD1 on nitrotyrosine levels. One-way ANOVA using Wistar rats (MCAO, MCAO + hyperglycemia, and SOD1 adenovirus MCAO + hyperglycemia) was used to determine the effect of hyperglycemia and SOD1 on blood glucose at baseline, MCAO, and reperfusion as well as myogenic tone for both ischemic and nonischemic vessels and infarct size, edema, beam walk, grip strength, and nitrotyrosine levels. SAS 9.3 (SAS, Cary, NC) was used for all analyses. Statistical significance was determined at α < 0.05, and a
Tukey’s post hoc test was used to compare means from significant ANOVAs.

RESULTS

Effect of hyperglycemia on myogenic tone. I/R impaired myogenic tone of MCAs isolated from both ischemic and nonischemic hemispheres compared with sham right and left hemispheres, respectively. When I/R injury was superimposed with acute hyperglycemia, myogenic tone impairment was exacerbated in the nonischemic side only. Interestingly, acute hyperglycemia alone reduced myogenic tone in MCAs isolated from the left side of sham hyperglycemic rats but had no effect on the right hemisphere (Fig. 1, A and B). In all groups, baseline blood glucose levels were similar. We achieved an acute elevation in blood glucose levels ranging between 200 and 250 mg/dl in the hyperglycemic group during MCAO and reperfusion (Fig. 1C).

Effect of global SOD1 overexpression on myogenic tone after acute hyperglycemic stroke. To determine the role of oxidative stress on decreased myogenic tone in hyperglycemic I/R injury, pressurized arteriography experiments were repeated in SOD1 transgenic rats. Since this model has a SD background, SD rats were used as proper controls. Short-term I/R impaired myogenic tone of MCAs obtained from normoglycemic SD rats compared with sham SD rats. MCAs isolated after I/R from normoglycemic and hyperglycemic SD rats displayed similar myogenic tone (Fig. 2A). SOD1 transgenic rats maintained a well-developed myogenic tone, similar to sham SOD1 rats after I/R with or without hyperglycemia (Fig. 2B). We achieved a similar reduction in CBF during MCAO in both SD and SOD1 rats. Percent CBF after reperfusion was improved in SOD transgenic rats exposed to MCAO, which was significantly reduced after acute hyperglycemic stroke (Fig. 2, D and E). SOD1 overexpression was confirmed by measuring SOD1 expression and activity in brain homogenates, which was significantly greater in brain homogenates of transgenic rats compared with wild-type SD rats (Fig. 3, A and B). In all groups, baseline blood glucose levels were similar and elevated at MCAO and reperfusion due to anesthesia. Hyperglycemic SD and SOD1 rats had higher blood glucose levels compared with normoglycemic groups at both time points (Fig. 3C). Nitrotyrosine levels, a marker of increased oxidative stress and peroxynitrite-mediated nitration, were significantly increased in SD rats after 30 min of ish-

![Graph A](image1.png)  ![Graph B](image2.png)  ![Graph C](image3.png)

**Fig. 1.** Acute hyperglycemia (HG) at the time of stroke exacerbated contralateral myogenic dysfunction. A: ischemia-reperfusion (I/R) with or without HG led to complete loss of the myogenic response in middle cerebral arteries (MCAs) isolated from the ischemic (IS) hemisphere compared with sham-operated (sham) right (RT) and sham RT with HG sides. B: the myogenic response was significantly reduced in the left (LT) hemisphere after I/R injury [nonischemic (NIS) side] or HG alone (sham LT + HG) compared with the sham LT side. Contralateral myogenic dysfunction was aggravated when I/R injury was superimposed with acute HG. *a,b,c* Different letters for pairs of means are significantly different ($P < 0.05$ by Tukey’s test, $n = 5–8$). C: blood glucose levels at baseline, MCA occlusion (MCAO), and reperfusion in sham, sham + HG, stroked Wistar (Wis MCAO), and Wis MCAO + HG rats. *#* $P < 0.001$ vs. sham and Wis MCAO rats ($n = 4$).
emia/24 h of reperfusion, whereas SOD1 overexpression prevented the elevation of nitrotyrosine levels after I/R with or without hyperglycemia (Fig. 3D).

**Effect of global SOD1 overexpression on neurovascular outcomes after acute hyperglycemic stroke.** Acute hyperglycemia led to infarct size and edema expansion in SD rats after 30 min of ischemia/24 h of reperfusion. Global SOD1 overexpression significantly reduced infarct size and edema compared with wild-type SD rats and prevented hyperglycemia-mediated increases in infarct size and edema (Fig. 4, A and B).

**Effect of global SOD1 overexpression on behavioral outcomes after acute hyperglycemic stroke.** Acute hyperglycemia worsened beam walk performance and induced grip strength deficits in SD rats after 30 min of ischemia/24 h of reperfusion. Hyperglycemic SOD1 transgenic rats displayed better beam walk performance and reduced grip strength deficits compared with hyperglycemic SD rats (Fig. 4, C and D).

**Effect of focal contralateral SOD1 overexpression on myogenic tone after acute hyperglycemic stroke.** Focal SOD1 overexpression in the MCA territory of the contralateral hemisphere significantly improved the myogenic response of vessels isolated from the nonischemic hemisphere but had no effect on the ischemic side compared with the effect of an empty vector injection and with MCAs isolated from rats exposed to acute hyperglycemic stroke (Fig. 5, B and C). Myogenic reactivity curves across pressure range under active and passive conditions for the nonischemic side showed a well-maintained autoregulatory response after focal SOD1 overexpression only (Fig. 5D). Passive vasodilation was significantly improved after contralateral SOD1 overexpression.
compared with the effect of an empty vector injection and with MCAs isolated from rats exposed to acute hyperglycemic stroke (Fig. 5E). To confirm contralateral SOD1 overexpression, SOD1 levels and activity were measured in brain homogenates. We found that SOD1 expression (Fig. 5F) and activity (Fig. 5G) were significantly upregulated in brain homogenates of the left injected hemisphere by nearly 50% compared with the right uninjected side, the left side injected with an empty vector, and control Wistar rats. In all groups, baseline blood glucose levels were similar. We achieved an acute elevation in blood glucose levels ranging between 200 and 250 mg/dl in hyperglycemic groups during MCAO and reperfusion (Fig. 5H).

**Effect of focal contralateral SOD1 overexpression on CBF after acute hyperglycemic stroke.** Mean CBF was significantly reduced in ischemic and nonischemic hemispheres, albeit to a different degree during MCAO and after reperfusion after acute hyperglycemic stroke compared with baseline (Fig. 6, A and B). Rats injected with SOD1 adenovirus 2 wk before acute hyperglycemic stroke displayed an improvement in CBF in both hemispheres after reperfusion (Fig. 6, A and B). We achieved a similar reduction in CBF during MCAO in both groups exposed to acute hyperglycemic stroke (Fig. 6C). Contralateral SOD1 overexpression increased CBF after reperfusion in both ischemic and nonischemic hemispheres after acute hyperglycemic stroke (Fig. 6D).

**Effect of focal contralateral SOD1 overexpression on neurovascular outcomes after acute hyperglycemic stroke.** Acute hyperglycemia significantly increased the infarct size and edema in Wistar rats after 30 min of ischemia/24 h of reperfusion. Contralateral SOD1 overexpression prevented hyperglycemia-mediated increases in infarct size and edema (Fig. 7, A and B).

**Effect of focal contralateral SOD1 overexpression on behavioral outcomes after acute hyperglycemic stroke.** Acute hyperglycemia worsened beam walk performance and induced grip strength deficits in Wistar rats after 30 min of ischemia/24 h of reperfusion. SOD1 overexpression in the contralateral hemisphere significantly improved neurological outcomes after acute hyperglycemic stroke (Fig. 7, C and D).

**DISCUSSION**

In the present study, we revealed a novel association between contralateral myogenic dysfunction and stroke outcomes after acute hyperglycemic stroke. Furthermore, we highlighted the critical role of SOD1 overexpression in improving vascular function and stroke outcomes. We provided evidence that
Contralateral myogenic dysfunction was exacerbated in hyperglycemia and was associated with poor stroke outcomes. We showed that improving vascular function specifically in the nonischemic hemisphere ameliorated neurovascular outcomes after acute hyperglycemic stroke. These findings are very important because they identify the contralateral hemisphere as a therapeutic target. Once the mechanisms and modulators of cerebrovascular function in both hemispheres are known, it will be possible to develop more effective strategies to deliver neuroprotective therapies to improve stroke outcomes and recovery.

Admission hyperglycemia (>7.8 mmol/l) is very common in ischemic stroke patients due to a history of diabetes or acute elevations in blood glucose (29). Both human and animal studies have reported that elevated blood glucose at stroke onset is associated with a larger infarct size, poor clinical outcomes, and an increased risk of mortality (2, 5, 7, 15). Until now, the only Federal Drug Administration-approved treatment for ischemic stroke is the intravenous administration of recombinant tissue plasminogen activator to restore blood flow (1). However, hyperglycemia at the time of ischemic stroke increases the risk of hemorrhagic transformation and poor clinical outcomes with recombinant tissue plasminogen activator administration (45). Moreover, several animal studies have shown that the exacerbated neurovascular injury in hyperglycemic stroke is more common with transient occlusion, suggesting that reperfusion contributes to increased brain damage by hyperglycemia (28, 39). However, the mechanisms by which acute hyperglycemia and diabetes aggravate vascular injury and neurological outcomes are multifactorial and still controversial (24, 28, 40). Since the only successful therapeutic target identified for the 800,000 annual victims of ischemic stroke is the cerebral vasculature (23), in our study, we focused on the impact of acute hyperglycemic reperfusion on cerebrovascular function. While diabetes also has a detrimental effect of cerebrovascular function and stroke outcomes, as recently reviewed (16, 26, 27), nondiabetic hyperglycemic patients may suffer the most from acute ischemic stroke compared with diabetic or normoglycemic patients (7, 23). As such, we narrowed our study to acute hyperglycemia.

The cerebrovascular myogenic response, discovered 100 yr ago by Bayliss (3), is the change in smooth muscle tone in response to pressure fluctuation. The myogenic response is an inherent property of smooth muscle cells that is crucial for maintaining vascular resistance and constant blood flow (31). Several experimental studies have shown a detrimental effect.
of I/R on the myogenic response of cerebral vessels isolated from ischemic hemispheres (9, 10). We recently showed in an animal model of transient MCAO, that I/R has a short-term global effect on cerebrovascular function. We reported that 30 min of MCAO/45 min of reperfusion reduced cerebral perfusion in both hemispheres and led to myogenic tone impairment in ischemic and contralateral hemispheres via increased peroxynitrite generation and nitration (11). The aim of the present study was to expand our previous findings by determining 1) the impact of admission hyperglycemia, a condition associated with poor outcomes, on myogenic tone in both hemispheres; 2) the role of oxidative stress on myogenic tone regulation in hyperglycemic stroke; and 3) the role of contralateral myogenic dysfunction reactivity on hyperglycemic stroke outcomes. We found that acute elevation of blood glucose at the time of stroke exacerbated myogenic dysfunction in MCAs isolated from the contralateral hemisphere, which was associated with infarct size expansion, increased edema, and poor neurological outcomes. However, acute hyperglycemia did not display any further effect on the myogenic reactivity of vessels isolated from the ischemic hemisphere compared with I/R alone. These results suggest that augmented contralateral myogenic dysfunction could contribute to poor outcomes after acute hyperglycemic stroke. Understanding this phenomenon

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Fig. 5. Contralateral SOD1 overexpression in control Wistar rats. Wistar rats were subjected to a stereotaxic injection of SOD1 adenovirus (AD) or an empty vector in the LT hemisphere 2–3 wk before acute hyperglycemic stroke. A: representative image of the stereotaxic injection of SOD1 AD in the contralateral hemisphere. B: focal contralateral SOD1 overexpression significantly improved contralateral myogenic dysfunction compared with NIS + HG and with acutely hyperglycemic stroked rats injected with an empty vector. C: contralateral SOD1 overexpression did not improve the myogenic response of vessels obtained from IS hemisphere (IS + HG SOD1 AD) after acute hyperglycemic stroke compared with IS + HG and with rats injected with an empty vector. a,b Different letters on pairs of means are significantly different (% < 0.05 by Tukey’s test, n = 3–6). D: focal contralateral SOD1 overexpression increased lumen diameter under active conditions while maintaining a well-developed autoregulatory response in the contralateral hemisphere compared with acute hyperglycemic stroke with and without the empty vector injection. E: focal contralateral SOD1 overexpression improved passive vasodilation in the contralateral hemisphere compared with the effect of an empty vector injection and with MCAs isolated from rats exposed to acute hyperglycemic stroke. *% < 0.001 vs. NIS + HG and NIS + HG empty vector (n = 3–6). F: SOD1 expression was significantly upregulated after stereotaxic injection of SOD1 AD in the LT hemisphere compared with the RT uninjected (uni) side, LT side injected with an empty vector, and control Wistar rats. *% < 0.05 vs. RT uninjected side; ***% < 0.01 vs. control and LT empty vector (n = 4–5). G: SOD activity was significantly upregulated after stereotaxic injection of SOD1 AD in the LT hemisphere compared with the RT uninjected side, LT side injected with an empty vector, and control Wistar rats. ***% < 0.001 vs. control, RT uninjected side, and LT empty vector (n = 3–6). H: Blood glucose levels at baseline, MCAO, and reperfusion in hyperglycemic stroked Wistar rats (Wis MCAO + HG), Wistar rats injected with SOD1 AD (SOD1 AD MCAO + HG), or Wistar rats injected with empty vector before hyperglycemic stroke (MCAO + HG empty vector) (n = 3–6).
is essential for the development of rational therapies that reduce hyperglycemic reperfusion injury in both hemispheres and thus improve clinical outcomes in patients.

Growing evidence indicates the involvement of oxidative stress in the pathogenesis of stroke through different mechanisms at neuronal and vascular levels (8, 36). Superoxide anion, a ROS, has emerged as an important mediator of vascular dysfunction (17). Besides its direct effect on blood vessels, superoxide is also the precursor for other ROS and some reactive nitrogen species. Considerable attention has been dedicated to the interaction of superoxide and nitric oxide, which reduces nitric oxide bioavailability, leading to peroxynitrite generation (44), which profoundly influences vascular function at multiple levels (33, 35). Our laboratory has previously reported the detrimental effect of excess peroxynitrite generation on myogenic reactivity in control and diabetic rats after I/R or short periods of oxygen-glucose deprivation (26). Another study (38) showed that MCAs perfused intraluminally with plasma of acutely hyperglycemic rats that underwent 2 h of MCAO/2 h of reperfusion experienced increased myogenic tone and that this was reversed by peroxynitrite decomposition. It is well established that ROS play a pivotal role in altering cerebrovascular function after I/R with or without hyperglycemia. Therefore, to test if depleting peroxynitrite parent radical (superoxide) could reverse myogenic dysfunction and hence improve stroke outcomes in our model of acute hyperglycemic stroke, we used SOD1 transgenic rats. We found that global SOD1 overexpression decreased brain nitrotyrosine levels (a marker for peroxynitrite generation), reserved myogenic behavior, and improved neurovascular injury after I/R with and without hyperglycemia compared with wild-type animals. These findings were in agreement with previous studies (25, 30, 37) that revealed the protective effect of SOD1 after cerebral ischemia. We then investigated whether improving myogenic tone only in the contralateral side could ameliorate poor neurovascular outcomes after acute hyperglycemic stroke. SOD1 expression was upregulated in the contralateral hemisphere using a stereotaxic injection of SOD1 adenovirus 2–3 wk before I/R. Our results showed that increased SOD1 activity in the contralateral hemisphere improved contralateral myogenic dysfunction after acute hyperglycemic stroke, which was associated with reduced infarct size and edema and better neurological performance. Several studies (19, 24, 25) have shown the protective role of SOD1 in improving stroke outcomes via modifying matrix metalloproteinase-9 activity, Akt activation, and others. Since we did not measure the effect of SOD1 overexpression on any of the previously proposed mechanisms in both hemispheres, we cannot conclude that the enhanced stroke outcomes are solely dependent on myogenic tone. However, the improvements in stroke outcomes after contralateral SOD1 overexpression seem to be due, in part, to the maintenance of a well-functioning myogenic response, as loss of contralateral myogenic tone was associated with poor neurovascular outcomes. These findings suggest that the nonischemic hemisphere may be a novel target for the management of stroke. The findings of the present study

Fig. 6. Contralateral SOD1 overexpression improved CBF after acute hyperglycemic stroke. Wistar rats were subjected to a stereotaxic injection of SOD1 AD or an empty vector in the LT hemisphere 2–3 wk before acute hyperglycemic stroke. A and B: mean percent CBF was significantly decreased in both hemispheres during MCAO and postreperfusion after acute hyperglycemic stroke compared with baseline. Contralateral SOD1 overexpression improved CBF after reperfusion in both IS and NIS hemispheres. C: percent CBF reduction during MCAO in IS and NIS hemispheres was similar between the two groups. D: contralateral SOD1 overexpression improved postreperfusion CBF in IS and NIS hemispheres after acute hyperglycemic stroke. *P < 0.001, **P < 0.01, and *P < 0.05 vs. baseline; ***P < 0.001 vs. postreperfusion and baseline; #P < 0.001 vs. IS + HG vehicle; ~P < 0.01 vs. NIS + HG vehicle (n = 5–6).
are limited by the fact that isoflurane was used as a method for anesthesia during stroke surgery, which can reduce myogenic reactivity. To normalize this effect, vessels were isolated from sham rats exposed to similar levels of isoflurane. However, these limitations do not outweigh the significant findings of the present study.

Stroke is the fourth leading cause of death and one of the major causes of permanent disability worldwide. The economic burden of stroke is significant, with the expected increase for stroke-related medical costs and disability from $71.6 to $184.1 billion between 2012 and 2030. Therefore, more detailed understanding of factors contributing to poor neurovascular outcomes and functional recovery will decrease the health and economic burdens of stroke. Several clinical studies have reported a reduction in CBF after stroke not only in the ischemic region but also in the contralateral hemisphere, known as cerebral diaschisis. Moreover, the persistent reduction of CBF in the hemisphere contralateral to the infracted region proved to be involved in poor outcomes and recovery (6, 41). In addition, various experimental and clinical studies have found major pathological changes in the contralateral hemisphere, including perivascular edema, blood-brain barrier damage, astrogliosis, and increased apoptosis after acute stroke (20, 34). Both clinical and experimental studies have suggested that pathological neurovascular changes in the contralateral hemisphere may contribute to stroke pathology and impede recovery processes. In agreement with the aforementioned studies, our findings showed that CBF was reduced in both hemispheres after acute hyperglycemic stroke, which was associated with poor neurovascular outcomes. Interestingly, we demonstrated that improving contralateral myogenic dysfunction in the contralateral hemisphere only was accompanied with an increase in CBF after reperfusion in both hemispheres, which led to better stroke outcomes. Although these results indicate the importance of a well-developed contralateral myogenic tone in improving functional outcomes after acute hyperglycemic stroke, this study was limited to the acute ischemic phase. Further studies are needed to demonstrate the impact of contralateral myogenic dysfunction at late ischemic stages. The role of the contralateral hemisphere in stroke outcomes and recovery is an intriguing but challenging new field of research. In our study, we highlight the importance of establishing effective clinical treatments that target both hemispheres to repair the injured vasculature close and distal to the site of ischemic injury to achieve better patient quality of life.

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Author contributions: M.C. and A.E. conception and design of research; M.C., W.L., S.H., and M.A. performed experiments; M.C. and M.H.J. analyzed data; M.C. interpreted results of experiments; M.C. prepared figures; M.C. drafted manuscript; M.C., S.C.F., and A.E. edited and revised manuscript; M.C., W.L., S.H., M.A., S.C.F., and A.E. approved final version of manuscript.

REFERENCES

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