Effects of Metformin in Experimental Stroke

Jun Li, PhD; Sharon E. Benashski, MS; Venugopal Reddy Venna, PhD; Louise D. McCullough, MD, PhD

**Background and Purpose**—Adenosine 5’-monophosphate-activated protein kinase (AMPK) is an important sensor of energy balance. Stroke-induced AMPK activation is deleterious because both pharmacological inhibition and genetic deletion of AMPK are neuroprotective. Metformin is a known AMPK activator but reduces stroke incidence in clinical populations. We investigated the effect of acute and chronic metformin treatment on infarct volume and AMPK activation in experimental stroke.

**Methods**—Male mice were subjected to middle cerebral artery occlusion after acute (3 days) or chronic (3 weeks) administration of metformin. Infarct volumes, AMPK activation, lactate accumulation, and behavioral outcomes were assessed. The roles of neuronal nitric oxide synthase and AMPK were examined using mice with targeted deletion of AMPK or neuronal nitric oxide synthase.

**Results**—Acute metformin exacerbated stroke damage, enhanced AMPK activation, and led to metabolic dysfunction. This effect was lost in AMPK and neuronal nitric oxide synthase knockout mice. In contrast, chronic metformin given prestroke was neuroprotective, improved stroke-induced lactate generation, and ameliorated stroke-induced activation of AMPK. Similarly, the neuroprotective effect of chronic prestroke metformin was lost in neuronal nitric oxide synthase knockout mice.

**Conclusions**—AMPK is an important potential target for stroke treatment and prevention. These studies show that the timing, duration, and amount of AMPK activation are key factors in determining the ultimate downstream effects of AMPK on the ischemic brain. *(Stroke. 2010;41:2645-2652.)*

**Key Words:** AMPK ■ cerebral ischemia ■ lactate ■ metformin

AMPK on the ischemic brain.

The downstream mediators of AMPK’s deleterious effects in the ischemic brain are not yet clear but may involve enhancement of lactic acidosis. Neurons, unlike astrocytes, have minimal activity of the key glycolytic enzyme PFK-2,5 have no glycogen stores,6 and are therefore exquisitely sensitive to hypoxia and hypoglycemia. Within a short period of energy deficiency, AMPK activation enhances astrocytic glycolysis and ketosis to provide energy to ischemic neurons.7 However, prolonged anaerobic glycolysis in astrocytes leads to progressive acidosis and inhibits the ability of neurons to use lactate as an energy source7,8 contributing to neuronal death. In cerebral ischemia, when energy depletion is severe, lactate acidosis enhanced by AMPK activation could lead to an exacerbation of stroke injury.

Metformin is a drug widely used for the treatment of Type 2 diabetes mellitus.9 It has been well documented that acute metformin treatment activates AMPK both in vivo and in vitro.10–12 AMPK activation is responsible for a number of the actions of metformin related to its glucose-lowering effects, including a decrease in glucose production in hepatocytes and increase in glucose uptake in skeletal muscle.12 The activation of AMPK by metformin requires nitric oxide (NO) because metformin no longer activates AMPK when NO is directly inhibited in bovine endothelial cells13 or in mice lacking the endothelial form of nitric oxide synthase.12 This suggests that activation of AMPK by metformin is NO-dependent, at least in the vasculature. Currently, there are no data on the effect of metformin on AMPK in the brain. Due to its AMPK-activating effects, treatment may exacerbate damage during acute ischemia. However, in clinical populations, chronic metformin treatment is associated with a lower risk of stroke, reducing cardiovascular mortality by
26%. This protection is independent of its glucose-lowering effect. The effects of chronic activation of AMPK in experimental stroke have not been previously investigated. In this study, we examined the effect of metformin in experimental stroke to determine effects on lactic acidosis, AMPK, and infarct size.

Materials and Methods
Focal Cerebral Ischemia Model and Physiology
The present study was conducted in accordance with National Institutes of Health guidelines for the care and use of animals in research and under protocols approved by the Center for Laboratory Animal Care at the University of Connecticut Health Center. Focal transient cerebral ischemia was induced in male mice (20 to 25 g) by right middle cerebral artery occlusion (MCAO) followed by reperfusion as described previously. At the end of ischemia (90 minutes MCAO except for chronic metformin post-stroke treatment experiments for which 60 minutes MCAO was used to minimize mortality), the animal was briefly reanesthetized, and reperfusion was initiated by filament withdrawal. In separate cohorts, blood glucose, physiological measurements, femoral arterial blood pressure, and cortical perfusion (laser Doppler flowmetry) were evaluated throughout MCAO and early reperfusion as described previously. Wild-type mice were purchased from Charles River (Wilmington, Mass). AMPK α2 knockout (KO) mice (C57BL/6 background) were obtained from Dr Benoit Viollet in France and rederived in house. Neuronal nitric oxide synthase (nNOS) KO mice (C57BL6 background) and corresponding littermates were obtained from Jackson Laboratories (Bar Harbor, Maine).

Behavioral Measurements
Neurological deficits (NDS) were scored in the intrasichemic period, 24 or 72 hours poststroke. The scoring system was as follows: 0, no deficit; 1, forelimb weakness and torso turning to the ipsilateral side when held by tail; 2, circling to affected side; 3, unable to bear weight on affected side; and 4, no spontaneous locomotor activity or barrel rolling.

Infarct Analysis
At 24 or 72 hours after stroke, the brain was removed and cut into 2-mm slices and stained with 1.5% 2,3,5-triphenyltetrazolium for 30 minutes at 3°C. Slices were formalin-fixed (4%) and then digitalized and infarct volumes analyzed (Sigma Scan Pro) as previously described. The final infarct volumes are presented as both direct volume (in millimeters) and indirect volume (percentage of contralateral structures with correction for edema). In animals assessed chronically poststroke, mice were euthanized at 3 weeks through a pentobarbital overdose and perfused transcardially with cold phosphate-buffered saline followed by 4% paraformaldehyde; the brain was postfixed for 18 hours and placed in cryoprotectant (30% sucrose). The brain tissue was cut into 40-μm free-floating sections on a freezing microtome and every eighth slice was stained by cresyl violet staining for evaluation of ischemic cell damage. Sections were digitized by a charged coupled device camera and analyzed with photoimaging software (Jandel Scientific). Due to the chronic nature of this study, cerebral atrophy was used as an indirect measure of cell loss. The volume of tissue atrophy was determined by measuring both hemispheres and lateral ventricles and transformed to millimeters cubed. Percent atrophy was computed by dividing the ischemic (right) hemisphere from the intact (left) hemisphere and then multiplying by 100 as in Lee et al.

Metformin Treatment
Metformin was dissolved in saline (vehicle). For acute treatment, metformin was injected daily for 3 days (50 or 100 mg/kg metformin or vehicle, intraperitoneally [IP]) before stroke. For chronic pre-stroke metformin treatment, wild-type and nNOS KO mice were injected for 3 weeks (50 mg/kg or vehicle) daily before stroke. Dose volume was 0.2 mL/20 g body weight. In an additional study, metformin (50 mg/kg per day IP) was administered 24 hours after the onset of MCAO for 3 weeks.

Western Blots
Western blots were done as described previously. Four hours after the onset of cerebral ischemia, mice were euthanized, brains were homogenized using lysis buffer, and protein was loaded on a 4% to 15% gradient sodium dodecyl sulfate–polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. p-AMPK was probed with antibody from Cell Signaling (1:1000). Beta-actin (1:5000; Sigma) was used as the loading control. Blots were incubated overnight in primary antibody at 4°C in Tris-buffered saline containing 4% bovine serum albumin and 0.1% Tween 20. Secondary antibodies (goat antirabbit IgG 1:5000 for p-AMPK, goat antimouse IgG 1:5000 for Beta-actin; Chemicon) were diluted and an electrochemiluminescence (Pico) detection kit (ThermoScientific) was used for signal detection.

Brain Lactate Measurement
Four hours after the onset of stroke, mice were euthanized and brains were homogenized with perchloric acid (6%) and spun; the supernatants were used for lactate measurements. Lactate measurement was done by colorimetric assay using a lactate assay kit following the manufacturer’s instructions (Abcam, Cambridge, Mass).

Statistics
Data were expressed as mean±SEM except for NDS, which was presented as median (interquartile range). Statistics were performed with Student’s t test, 1-way analysis of variance with Tukey post hoc test for multiple comparisons (for p-AMPK), or by Mann–Whitney U test (NDS). A probability value <0.05 was considered to be statistically significant. Investigators performing MCAO, behavioral, and infarct size analysis were blinded to treatment group.

Results
Acute Metformin Treatment Increased Infarct 24 Hours After Stroke
Twenty-four hours after MCAO, metformin (100 mg/kg) significantly increased total (metformin 67.0%±6.8% versus vehicle 42.8%±4.2%; P<0.05), cortical (metformin 66.8%±5.8% versus vehicle 42.2%±4.6%; P<0.05) as well as striatal (metformin 77.4%±4.5% versus vehicle 57.6%±3.3%; P<0.05) infarct volume (direct infarct volumes mm3; cortex—metformin: 67.8±7.1 versus vehicle: 38.5±6.0, P<0.05; striatum—metformin: 33.8±0.92 versus 21.9±2.3, P<0.05; total—121±9.8 versus 70.4±7.5, P<0.05). At 50 mg/kg, metformin increased both total (metformin 61.2%±4.7% versus vehicle 42.8%±4.2%; P<0.05) and striatal (metformin 75.1%±5.6% versus vehicle 57.6%±3.3%; P<0.05) infarction volume but had no significant effect on cortical infarction (metformin 56.9±7.8 versus vehicle 42.2%±4.6%) compared with vehicle-treated mice (Figure 1A; direct infarct volumes mm3; cortex—metformin: 56.8±7.5 versus vehicle: 38.5±6.0; striatum—metformin: 32.2±2.0 versus 21.9±2.3, P<0.05; total—113±9.3 versus vehicle 70.4±7.5, P<0.05). The detrimental effect of metformin was also reflected in the NDS (metformin 100 mg/kg: 3.0 [0] versus vehicle 2.0 [1]; P<0.05; Supplemental Table I, available at http://stroke.ahajournals.org). No mortality was seen in any of the groups.

Acute Metformin Treatment Increased Infarct 72 Hours After Stroke
In the 72-hour survival group, metformin (100 mg/kg·L−1) also significantly increased total (metformin 66.0%±2.2% versus...
vehicle 49.6% ± 5.1%; P < 0.05), striatal (metformin 74.4% ± 2.3% versus vehicle 61.0% ± 4.5%; P < 0.05) as well as cortical (metformin 58.9% ± 1.9% versus vehicle 43.1% ± 7.2%; P < 0.05) infarct volume (contralateral hemisphere) in comparison to the saline-treated group (Figure 1B; direct infarct volumes mm³; cortex—metformin: 51.2 ± 1.8 versus vehicle: 41.2 ± 5.1; striatum—metformin: 39.4 ± 3.9 versus 27.3 ± 1.4, P < 0.05; total—104 ± 4.8 versus 79.2 ± 8.2, P < 0.05). Neurological deficits were also exacerbated by metformin treatment (metformin 3.0 [1] versus vehicle 2.0 [0]; P < 0.05; Table 1).

There were no differences in physiological measurements between the acute or chronic metformin and vehicle-treated groups (Supplemental Table II). Laser Doppler flowmetry was equivalently reduced during ischemia and was restored equally in early reperfusion (Table 2). The mortality rates were equivalent between groups (1 of 11 in vehicle and 2 of 13 in metformin).

**Acute Metformin Increased pAMPK Levels**

As expected, stroke significantly induced pAMPK levels 4 hours after the onset of stroke (stroke 1.63 ± 0.19 versus sham 0.87 ± 0.079, P < 0.05, n = 5). Metformin administration (100 mg/kg) significantly enhanced stroke-activated pAMPK levels compared with vehicle-treated MCAO mice (metformin 3.02 ± 0.36 versus vehicle 1.63 ± 0.19, P < 0.05; Figure 2A, B).

**Deletion of AMPK Abolished the Deleterious Effects of Acute Metformin**

To investigate if metformin produces its detrimental effects specifically through AMPK activation, we examined the effect of acute metformin in AMPKα2 knockout mice. The deleterious effects of acute metformin treatment were ameliorated in AMPKα2−/− mice (cortex—vehicle 38.5% ± 3.4% versus metformin 35.6% ± 5.8%; striatum—vehicle 52.6% ± 5.9% versus metformin 60.0% ± 7.6%; total—vehicle 33.3% ± 3.2% versus metformin 33.4% ± 4.1%; Figure 3A; direct infarct volumes mm³; cortex—vehicle: 34.5 ± 2.1 versus metformin 32.0 ± 5.5; striatum—vehicle 18.0 ± 3.4 versus metformin 19.3 ± 2.7; total—vehicle 55.2 ± 3.9 versus metformin 60.7 ± 7.9). No mortality was seen in the groups.

**AMPK Mediates Its Effects Through NO**

It has been shown that activation of AMPK by metformin is dependent on NO in the vasculature. The detrimental effects of acute metformin treatment were lost in male nNOS−/− mice (cortex—vehicle 31.4% ± 5.0% versus metformin 30.5% ± 4.8%; striatum—vehicle 41.2% ± 6.5% versus 44.8% ± 7.9%; total—vehicle 34.4% ± 5.2% versus metformin 32.3% ± 5.0%; Figure 3B; direct volume mm³; cortex—27.4 ± 5.0 versus 32.2 ± 4.9; striatum—18.3 ± 3.7 versus 19.9 ± 3.4; total—54.1 ± 9.1 versus 54.3 ± 7.9). The mortality rates were not significantly different between groups (0 of 10 in vehicle and 1 of 12 in metformin).

**Stroke-Induced Lactate Levels Were Elevated in Mice Acutely Treated With Metformin**

Brain lactate levels were increased 4 hours after stroke (vehicle stroke 15.1 ± 0.36 versus vehicle sham 9.62 ± 0.19, P < 0.05, n = 4/pg). Acute metformin treatment significantly exacerbated the stroke-induced increase in lactate levels.

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**Table 1. Effects of Acute or Chronic Metformin Treatment on NDS After MCAO**

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*For acute treatment, metformin was injected to mice for 3 days before stroke at a dose of 100 mg/kg and the NDS was collected at either 24 hours or 72 hours after stroke. For chronic treatment, metformin was given for 3 weeks daily (50 mg/kg) and neurological deficit scores were collected 24 hours after stroke.

†P < 0.05 versus control (Mann–Whitney U test); data are expressed as median (interquartile range).
metformin versus vehicle (1-way analysis of variance with Tukey post hoc test); data are expressed as mean±SEM. *For acute treatment, metformin (100 mg/kg) was administered (IP) for 3 days before MCAO (A). For chronic treatment, metformin (50 mg/kg) was administered (IP) for 3 weeks before MCAO (B). Blood samples were collected from femoral arteries at the onset of MCAO or 60 minutes from the onset of MCAO. All the data are shown as mean±SEM (n=4). Student t test was used to compare the difference of each parameter between the metformin- and saline-treated mice. P<0.05 is considered to be significant statistically.

LDF indicates Local Cerebral Blood Flow; MABP, mean arterial blood pressure.

Chronic Prestroke Treatment With Metformin Was Neuroprotective

Chronic treatment with metformin reduced infarct size 24 hours after stroke both in the striatum and total hemisphere (cortex—48.3±1.1% versus 32.1±6.6%; striatum—53.6±4.0% versus 45.3±2.9%, P<0.05; total—45.3±2.9% versus 29.3±4.9%, P<0.05; Figure 5; direct volumes mm³; cortex—46.9±2.6 versus 29.7±6.9; striatum—25.6±2.6 versus 12.4±1.9; total—82.8±6.1 versus 49.9±9.2, P<0.05). The protective effect of chronic metformin treatment was reflected in the NDS (metformin 1.5 [1] versus vehicle 2.0 [1]; P<0.05; Table 1). The mortality rates were equivalent between groups (1 of 7 in vehicle and 1 of 9 in metformin).

The Effect of Chronic Prestroke Metformin Treatment Was Mediated by NO

Because NO may play a role in metformin’s effect in stroke, we tested the effect of chronic metformin treatment in nNOS

Figure 2. Acute metformin treatment activated AMPK after stroke. Brains were collected 4 hours after stroke. Vehicle/metformin (100 mg/kg) was administered through IP for 3 days before MCAO. n=5 /pg. *P<0.05 stroke versus sham, #P<0.05 metformin versus vehicle (1-way analysis of variance with Tukey post hoc test); data are expressed as mean±SEM.
KO mice. Chronic Metformin treatment (prestroke) lost its neuroprotective effect in nNOS KO mice suggesting that this effect is mediated by nNOS (cortex—metformin 38.4% ± 4.6% versus vehicle 32.5% ± 3.4%; striatum—metformin 45.6% ± 3.4% versus 49.0% ± 5.1%; total—metformin 35.8% ± 2.0% versus 32.0% ± 2.7%; Figure 6; direct volume mm\(^3\); cortex—29.8 ± 2.2 versus 26.9 ± 3.4; striatum 18.6 ± 2.3 versus 16.2 ± 2.8; total—53.8 ± 4.1 versus 52.8 ± 6.1). Mortality rate was 1 of 7 in each group.

Chronic Prestroke Treatment With Metformin Downregulated pAMPK and Ameliorated Lactate Accumulation

Chronic treatment with metformin before stroke reduced pAMPK level in stroke when compared with sham metformin-treated mice (Figure 7A, B). As expected, lactate levels after stroke were elevated in animals treated with vehicle. However, chronic administration of metformin led to an amelioration of stroke-induced lactate generation because levels were not significantly different from metformin-treated sham animals (Figure 7C).

Chronic Prestroke Treatment With Metformin Downregulated pAMPK and Ameliorated Lactate Accumulation

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Discussion

This article demonstrates several important new findings regarding the role of AMPK in stroke. First, acute metformin activates AMPK in the brain in vivo as documented by elevations in pAMPK in brain homogenates. Second, metformin-induced AMPK activation specifically exacerbates stroke damage because the detrimental effects of acute metformin are abolished in AMPK \(\alpha_2\) mice, the catalytic isoform that is responsible for the detrimental effect of AMPK activation in ischemic brain. Third, the signaling pathway involves NO. Both acute metformin-induced exacerbation of ischemic damage and the neuroprotection induced by chronic metformin treatment were lost in nNOS KO mice. Interestingly, the protective effect of chronic metformin had a long therapeutic window because a neuroprotective effect was seen even if treatment was not initiated until 24 hours after the onset of stroke. Acute metformin administration significantly increased brain lactate levels after stroke, which may represent increased anaerobic glycolysis secondary to AMPK activation. Finally, chronic prestroke metformin treatment was neuroprotective, was associated with less stroke-induced AMPK activation, and ameliorated the detrimental metabolic changes seen with acute metformin administration. This suggests that the duration and degree of AMPK activation are critical factors in determining the downstream physiological response in the ischemic brain.

The role of AMPK in stroke has been a subject of considerable debate. Several studies, both in vivo and in vitro, have demonstrated that acute AMPK activation is detrimental, whereas others have suggested that it may play a neuroprotective role. Stroke represents a state of severe energy deficiency, because there is no available substrate for the energy consumptive pathways activated by AMPK. We have previously shown that the AMPK activator AICAR
exacerbated stroke injury; however, AICAR has numerous “off-target” effects that could contribute to enhanced injury, that is, vasodilatation through activation of adenosine.3 Because metformin is also known to activate AMPK, yet has clear beneficial effects on stroke incidence in clinical populations, we wanted to determine the effect of AMPK in an acute stroke model, recognizing that stroke incidence and acute neuroprotective effects may be mediated by different mechanisms. Surprisingly, the effect of metformin in the ischemic brain appears to be dependent on duration of treatment and is linked to activation of AMPK.

There are several possible mechanisms by which metformin can activate AMPK. Metformin can produce a decline in the free adenosine 5′-triphosphate/adenosine 5′-diphosphate and adenosine 5′-triphosphate/AMP ratios serving as a stimulus for AMPK phosphorylation and activation.10 Alternatively, metformin can directly activate an upstream AMPK kinase or promote AMPK phosphorylation by binding to AMPK, making it a better substrate for an AMPK kinase.18 Xie et al has found that LKB1, an AMPK kinase, is required for metformin-induced AMPK activation.19 Metformin increased the phosphorylation and nuclear export of LKB1 into the cytosol in endothelial cells, leading to enhanced association of AMPK with LKB1. Inhibition of LKB1 abolished the stimulatory effect of metformin on AMPK, and conversely, LKB1 overexpression enhanced metformin-induced AMPK phosphorylation.19 There are little data on possible interactions between LKB1 and NO in the brain. However, it is known that NO reacts with superoxide anions to form the potent oxidant peroxynitrite, which activates AMPK in a LKB1-dependent manner in cultured endothelial cells.20 It is possible that in cerebral ischemia, where oxidative stress is high, metformin activates NO, forming peroxynitrite, leading to activation of AMPK. However, in this study, we found no differences in LKB1 phosphorylation or translocation in metformin-treated animals (data not shown).

Our data demonstrate that the AMPK activation induced by metformin requires NO, because acute metformin treatment has no effect in male nNOS KO mice after stroke. It is well known that among the 3 major NO synthase isoforms in the brain, nNOS is the primary isoform in neurons.21 Our studies suggest that the detrimental effects of acute metformin are mediated in part by activation of nNOS. However, it is well known that AMPK, like NOS, is expressed in neurons, astrocytes, and endothelium.1,3,22 Our studies evaluated stroke outcome in an in vivo model, in which all cell types interact “in situ” and assessed AMPK activation in whole brain homogenates; therefore, we cannot definitively exclude effects of metformin in the endothelium or other cell types. Although it is likely that the activation of AMPK by acute metformin in the neurons produced the detrimental effect of this drug in ischemia, further confirmation of this in vivo will require examination of mice with neuron-specific deletion of AMPK, which is currently under development.

The mechanisms through which acute AMPK activation exacerbates stroke injury are not yet clear. Studies have suggested exacerbated lactate accumulation, autophagy, and increased glucose due to unregulated glucose transporters in the reperfusion phase may contribute to stroke damage (for review, see Li et al17). AMPK can increase glycolysis by activating PFK-2, increasing fatty acid oxidation, activating glucose transport (through glucose transporter 4), and inhibiting glycogen synthesis to enhance available energy to the deprived cell.23 However, neurons are known to have minimal anaerobic glycolytic capacity.5 Astrocytes can perform glycolysis; however, overactivation of astrocytic glycolysis can be detrimental. After ischemia in the brain, astrocytic PFK-2 activation is increased stimulating PFK-1, a glycolytic regulatory enzyme, subsequently leading to the production of

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**Figure 7.** Effect of chronic metformin on AMPK phosphorylation and lactate levels after stroke. Metformin was given to wild-type mice at 50 mg/kg daily for 3 weeks before stroke. A–B, Metformin-treated mice had reduced pAMPK levels after stroke when compared with sham mice treated with metformin (n=2 in sham and n=3 stroke). Brains were removed 4 hours after stroke and homogenates were used for Western blots. Statistics were performed with Student t test. *P<0.05 versus metformin sham. C, Metformin chronic treatment did not enhance lactate accumulation in stroke. Brains were removed 4 hours after stroke and homogenized in perchloric acid (6%). Lactate was immediately measured using the Lactate Assay Fluorometric Kit (Abcam). n=4/group. Statistics were performed with 1-way analysis of variance with Tukey post hoc test, *P<0.05 versus vehicle sham; data are expressed as mean±SEM.
Lactate. Lactate can also be produced directly from glycogen, which astrocytes store in large amount. These processes result in progressive acidosis. Lactic acid is more cytotoxic than inorganic acids such as hydrochloric acid because it generates further intracellular acidosis and induces cellular edema. In clinical settings, it has been well described that, although rare, metformin can cause lactic acidosis in the periphery and the underlying etiology for this is not clear.

As an initial step to investigate the mechanism by which acute metformin exacerbates injury after MCAO, we examined the effect of acute metformin on cerebral lactate levels. Acute metformin significantly enhanced stroke-induced lactate accumulation, suggesting that lactate may contribute to the detrimental effect of acute metformin. However, it is possible that the increased lactate may simply reflect the larger infarct volume. Because the accumulation occurs quite early after ischemia, it is likely that the increased lactate is in part responsible for the detrimental effects of acute metformin. It is noteworthy that lactate levels in the chronic metformin-treated sham group are slightly higher than the vehicle sham group, suggesting long-term activation of AMPK may enhance lactate in the intact, noninjured, brain. Metformin may serve as a preconditioning stimulus, making the brain less vulnerable to subsequent injury by exposing it to low levels of metabolic stress.

Chronic treatment with metformin reduced infarct volume, reduced stroke-induced lactate generation, and decreased stroke-induced AMPK activation. Because we have shown that acute AMPK activation is detrimental after MCAO, chronic activation could lead to a downregulation of AMPK and hence neuroprotection. Our data demonstrated that the neuroprotective effect of chronic metformin was lost in nNOS KO mice. This suggests that neuronal NO plays an important role in both the deleterious effects of acute metformin and the beneficial effects of chronic metformin. Importantly, chronic metformin treatment reduced tissue atrophy measured 3 weeks after stroke, even when treatment was delayed for 24 hours. This suggests that continued cell death occurs after MCAO and that this penumbra is potentially salvageable by manipulations of energy-sensing mechanisms. The mechanism for this effect is unclear but may involve reductions in poststroke inflammation, vascular dysfunction, or metabolic derangements, or alternatively, enhancement of poststroke growth factors or postconditioning, many of which are intricately tied to alterations in AMPK activity.

Activation are key factors in determining the ultimate downstream effects of AMPK on the brain. The finding that acute activation of AMPK increased ischemic damage further confirms that acute AMPK activation is detrimental in stroke, consistent with previous findings from other pharmacological and genetic studies. The detrimental effect of acute AMPK activation may be mediated, at least in part, by enhancement of lactic acidosis. Chronic metformin treatment may lead to sublethal metabolic stress and downregulate AMPK protecting the brain from subsequent injury.

Sources of Funding
This work was supported by National Institutes of Health grants R01 NS050505 and NS055215 (to L.D.M.) and American Heart Association grant 09SDG2261435 to J.L.L.

Disclosures
None.

References


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Stroke. 2010;41:2645-2652; originally published online September 16, 2010;
doi: 10.1161/STROKEAHA.110.589697
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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Table 1. Effects of acute or chronic Metformin treatment on neurological deficit scores (NDS) after MCAO. For acute treatment, Metformin was injected to mice for 3 days prior to stroke at dose of 100 mg/kg and the NDS was collected at either 24 hours or 72 hours after stroke. For chronic treatment, Metformin was given for 3 weeks daily (50 mg/kg) and neurological deficit scores were collected 24 hours after stroke. * P<0.05 versus control (Mann-whitey U-test); data are expressed as median (interquartile range).

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Table 2. No differences were seen in any physiological parameters, blood glucose, or local cerebral blood flow (LDF) between acute or chronic Metformin treated and vehicle treated mice. For acute treatment, Metformin (100 mg/kg) was administered (i.p.) for 3 days prior to MCAO (Table 2A). For Chronic treatment, Metformin (50 mg/kg) was administered (i.p.) for 3 weeks prior to MCAO (Table 2B). Blood sample were collected from femoral artery at the onset of MCAO or 60 min from the onset of MCAO. All the data are shown as mean±sem (n=4). Student’s t-test was used to compare the difference of each parameter between the Metformin and saline treated mice. P<0.05 is considered to be significant statistically.

Table. 2A

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<td>pO2</td>
<td>Glucose</td>
<td>MABP(m mHg)</td>
<td>LDF % base level</td>
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