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Uncoupling of neurovascular communication after transient global cerebral ischemia is caused by impaired parenchymal smooth muscle K_{ir} channel function

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Abstract

Transient global cerebral ischemia is often followed by delayed disturbances of cerebral blood flow, contributing to neuronal injury. The pathophysiological processes underlying such disturbances are incompletely understood. Here, using an established model of transient global cerebral ischemia, we identify dramatically impaired neurovascular coupling following ischemia. This impairment results from the loss of functional inward rectifier potassium (K_{IR}) channels in the smooth muscle of parenchymal arterioles. Therapeutic strategies aimed at protecting or restoring cerebrovascular K_{IR} channel function may therefore improve outcomes following ischemia.

Keywords

Neurovascular coupling, cerebral blood flow, ischemia, K_{IR} channel, smooth muscle

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Introduction

Local cerebral blood flow (CBF) is under dynamic regulation to match neuronal activity, ensuring adequate oxygen and nutrient delivery at all times.[1] This process—neurovascular coupling (NVC)—is coordinated by the neurovascular unit (neurons, astrocytes, vascular smooth muscle, and endothelial cells) and forms the basis of functional hyperemia. Multiple mechanisms have been proposed to mediate NVC, and the relative contribution of each remains a matter of debate.[2–6] A widely held view is that astrocytes respond to neuronal activation with an intracellular calcium (Ca^{2+}) wave that engages Ca^{2+}-dependent pathways in astrocytic endfeet enwrapping parenchymal arterioles (PAs), causing the release of vasodilatory substances.[3,7] Among the various vasodilators implicated, potassium (K^+) released through astrocytic large-conductance Ca^{2+}-activated K^+ (BK) channels is a major contributor.[8–10] Increased perivascular K^+ activates inward rectifier K^+ (K_{IR}) channels expressed on PA smooth muscle cells (SMCs), driving membrane hyperpolarization and vasodilation.[2,9]

Disruption of brain blood supply results in global cerebral ischemia, the most common cause of which is cardiac arrest (CA).[11–13] Although stable cardiac rhythm may be restored by resuscitation, the post-arrest period is often associated with disturbances in CBF.[11,12,14–16] The mechanisms underlying such disturbances remain unknown. Here, using a rat model of transient global cerebral ischemia (TGCI), we found that K_{IR} currents were depressed in PA myocytes after ischemia, rendering arterioles unable to respond

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to vasodilatory K^+ ions released during neuronal activity. This uncoupling of NVC may cause functional ischemia in the days following an ischemic event, thereby worsening neurological damage.

Materials and methods

Animals

Male Sprague-Dawley rats (~250–300 g; Charles River, Canada) were kept on a 12-h light:dark cycle with free access to food and water. Procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health policy on the care and use of laboratory animals. Animals were randomly allocated to undergo TGCI or sham surgery and separate sets of animals underwent surgery for each type of experiment. Since the majority of experiments were performed by the surgeon, blinding was not possible. The same number of animals in each group underwent surgery for each type of experiment, but the final reported number of animals differed due to attrition. Results are reported in compliance with the ARRIVE guidelines.

TGCI

Reversible forebrain ischemia was induced by a 15-min occlusion of both carotid arteries combined with concomitant hypovolemia.[17] Rats were anaesthetized with 3.5% isoflurane (Abbott Laboratories, USA) in atmospheric air/O_2 (70:30), orally intubated, and artificially ventilated with 1.5–2% isoflurane. A catheter was inserted in the tail artery for blood pressure recording, gas analysis, and infusions. Body temperature was maintained at 37°C. Muscle relaxation was achieved by i.v. injection of 0.2 mg/ml bolus doses (0.2 ml) of Norcuron (Merck, USA) every 10 min. A catheter filled with heparin was inserted via the external jugular vein into the right atrium. Rats received 0.5 ml heparin (100 IU/ml) and were allowed to equilibrate for 15–20 min. Ischemia was induced by lowering mean arterial blood pressure to 40 mm Hg by withdrawing blood through the jugular vein catheter, followed by bilateral clamping of both common carotid arteries for 15 min. Thereafter, clamps were released and normal blood pressure was restored by reinfusion of blood. Systemic acidosis was counteracted by i.v. injection of 0.5 ml of 0.6 M sodium bicarbonate. Sham-operated animals underwent the same procedure without carotid clamping and blood pressure lowering.

Brain slices

Rats were euthanized by i.p. injection of sodium pentobarbital. The brain was removed into ice-cold artificial cerebrospinal fluid (aCSF) consisting of 124 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 4 mM glucose. Slices (160–190 μm) were prepared using a Leica VT 1000S vibratome (Leica Biosystems, USA) then loaded with 10 μM Fluo-4 AM (Invitrogen, USA) in aCSF containing 2.5 μg/ml pluronic acid for 1.5 h at 32°C. Measurement of arteriolar diameter in response to electrical field stimulation (EFS) and Ca^{2+} imaging was performed as previously described.[18] To quantify endfoot $[Ca^{2+}]_i$, we treated some slices with 10 μM ionomycin and 20 mM $[Ca^{2+}]_o$ to obtain a maximal fluorescence measurement. We concluded all other experiments by perfusing with aCSF containing 50 μM diltiazem, 200 μM papaverine, 0 $CaCl_2$, and 5 mM EGTA to obtain the maximal vessel diameter. The intensity and pulse pattern of EFS (a 3 s train delivering a 20 V, 50 Hz alternating square pulse of 0.3 ms duration) remained constant throughout all experiments.

Pressure myography

Brains were removed and placed in ice-cold MOPS-buffered PSS (pH 7.4) consisting of 3 mM MOPS, 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1 mM KH_2PO_4, 5 mM glucose, and 1% bovine serum albumin. Cortical PAs were pressurized to 40 mm Hg in a Living Systems Instrumentation (USA) arteriograph and continually perfused with aCSF. Inner lumen diameter was measured using IonWizard 6.1 software (IonOptix, USA). For solutions in which $[K^+]$ was raised, osmolarity was maintained by exchanging NaCl for KCl.

Electrophysiology

Arterioles were incubated in a solution of 55 mM NaCl, 80 mM Na-glutamate, 6 mM KCl, 2 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES (pH 7.3) containing 0.3 mg/ml papain (Worthington, USA) and 0.3 mg/ml dithioerythritol (DTE) for 14 min at 36°C, and then transferred to a solution of the same composition (minus papain and DTE) containing 1 mg/ml collagenase F and 100 μM $CaCl_2$ for 5 min at 36°C. SMCs were dispersed by trituration. Myocytes were patch clamped in the whole-cell perforated configuration, and currents were amplified using an Axopatch 200B amplifier. Currents were filtered at 2 kHz and digitized at 20 kHz. Pipettes were fabricated from borosilicate glass (1.5 mm o.d., 1.17 mm i.d; Sutter Instruments, USA), and fire-polished to give a tip resistance of $<3\,M\Omega$ then filled with a solution consisting of 10 mM NaCl, 110 mM K-aspartate, 30 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, and 250 μg/mL amphotericin B (pH 7.2). The bath solution consisted of

80 mM NaCl, 60 mM KCl, 10 mM HEPES, 4 mM glucose, and 100 μM $CaCl_2$ (pH 7.4). Low bath Ca^{2+} was used to minimize the contribution of BK channel activity to recorded currents, and current subtraction was used to isolate Ba^{2+}-sensitive K_{IR} currents for further analysis. Cell capacitance was identical between groups.

Data are expressed as means ± S.E.M., and $P \leq 0.05$ was considered significant. Statistical tests are noted in figure legends. Drugs and chemicals were obtained from Sigma-Aldrich (USA) unless otherwise stated.

Results

NVC is severely reduced 48 h after TGCI

Brain slice arterioles lack the normal hemodynamic properties of blood flow and intravascular pressure and therefore display no myogenic tone.[19] To measure dilatory responses to neuronal activity evoked by EFS, we mimicked physiological tone in slice arterioles by pre-constricting them with the thromboxane analog 9,11-dideoxy-9α,11α-methanoepoxy $PGF_{2\alpha}$ (U46619; 125 nM). This constricted arterioles in slices from both sham and ischemic rats to the same degree (Figure 1(a)). At two days after TGCI, EFS evoked 16% ± 3% dilation in ischemic rats, compared to a 58% ± 9% dilation in sham animals, indicating that NVC is attenuated after ischemia (Figure 1(b) to (d)).

Astrocytic endfoot Ca^{2+} handling is unaffected by TGCI

A key signaling step in NVC is an increase in astrocytic endfoot Ca^{2+} concentration ($[Ca^{2+}]_i$), which drives the release of vasoactive factors onto the underlying vascular SMCs of PAs, causing vasorelaxation and increased blood flow (27). Thus, deficits in NVC might result from deranged astrocytic endfoot Ca^{2+} handling. To address this possibility, we quantified endfoot $[Ca^{2+}]_i$ evoked by EFS.[10,18] We detected no difference in either the temporal characteristics of the endfoot Ca^{2+} wave (Figure 1(e)) or resting or evoked $[Ca^{2+}]_i$ (Figure 1(f)), suggesting that the NVC deficit induced by TGCI resides in the arteriole.

PA dilation to extracellular K^+ is diminished by TGCI

During NVC, K^+ is released from astrocytic endfeet in response to Ca^{2+} elevations and contributes to the vasodilation that facilities hyperemia by activating SM K_{IR} channels.[9] Diminished NVC in the absence of a difference in endfoot Ca^{2+} handling after ischemia suggests that this defect could reflect impairment of the ability of PAs to sense and respond to extracellular K^+. To test this, we measured PA diameter in brain slices from sham and ischemic rats in response to an increase in bath K^+ from 3 mM to 10 mM. Consistent with an impairment of PA K_{IR} channels, PAs from TGCI rats demonstrated blunted dilations to 10 mM K^+ (Figure 1(g) and (h)).

Because brain slice observations can be confounded by the presence of multiple cell types, and arterioles in this preparation lack physiological pressure, we chose to test the responsiveness of isolated, pressurized (40 mm Hg) PAs to step-wise increases in K^+ from 3 mM to 8, 15, 20, 30, and 60 mM. In PAs from shams (Figure 2(a)), small increases in $[K^+]_o$, to <20 mM, evoked rapid and substantial vasodilation, attributable to the activation of sarcolemmal K_{IR} channels,[2,20] and deactivation of L-type voltage-dependent Ca^{2+} channels (VDCCs) through membrane potential hyperpolarization. Further increases in $[K^+]_o$ evoked substantial vasoconstriction through membrane depolarization and activation of L-type VDCCs.[20,21] In arterioles from ischemic rats (Figure 2(b) and (c)), the vasoconstrictor response to high concentrations of K^+ was unaffected, but the vasodilatory response to small increases was significantly attenuated at 10 and 15 mM $[K^+]_o$ and trended lower in response to an increase from 3 mM to 8 mM $[K^+]_o$.

Ischemia downregulates PA SM K_{IR} currents

PA dilations to K^+ are mediated by K_{IR} channels.[9] Strikingly, isolated PA SMCs from ischemic animals displayed significantly smaller barium (Ba^{2+}; 100 μM)-sensitive K_{IR} currents than their control sham-operated counterparts (Figure 2(e) and (f)), suggesting that ischemia leads to reduction in the number of functional K_{IR} channels in the PA myocyte membrane.

Discussion

TGCI is often followed by a reduction in CBF termed "delayed post-ischemic hypoperfusion" (DPH), which contributes to neuronal cell death.[22–25] Most studies investigating perfusion deficits following global cerebral ischemia have focused on changes in global CBF and the larger cerebral arteries, and/or the neuronal consequences of DPH.[24,26,27] In contrast, changes in the microvasculature and NVC have received little attention.

Employing a well-characterized model of TGCI,[17] we made the striking observation that NVC was almost abrogated two days after ischemia. Because astrocytic Ca^{2+} signaling remained normal, this impairment of NVC is probably not attributable to reduced release of Ca^{2+}-dependent vasodilatory substances from astrocytic endfeet and instead suggests a possible defect in the PA SMCs that respond to released vasodilator substances. Our data strongly support this hypothesis, showing that dilation of PAs to small

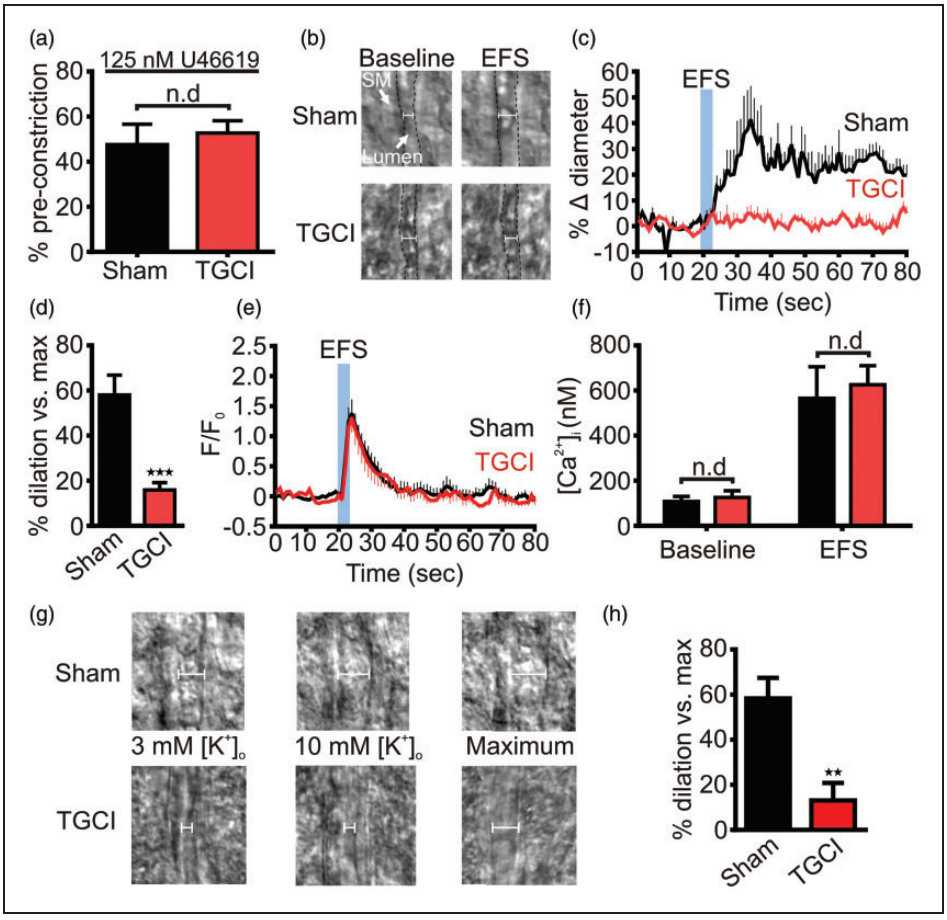

Figure 1. Transient global cerebral ischemia disrupts K^+-mediated neurovascular coupling in brain slices. (a) U46619 (125 nM) evoked the same degree of constriction in rat parenchymal arterioles in brain slices from sham (48% ± 9%; $n = 5$) and ischemic rats (53% ± 5%; $n = 5$). This maneuver simulates basal myogenic tone in the absence of intravascular pressure. (b) EFS evoked substantial arteriolar dilation in brain slice arterioles from sham, but not TGCI, rats. Lumen edges are indicated by the dashed black line. SM: smooth muscle. (c) Averaged time-courses showing brain slice arteriolar diameters before and after EFS. In sham rats ($n = 7$) EFS produced robust vasodilations, whereas after TGCI ($n = 8$) this response was absent; 10 animals were used for each group, the lower n numbers reported reflect attrition from unsuccessful experimental preparations. (d) Summary dilations indicating the vasodilation relative to passive diameter of the arteriole. Peak dilations were substantially blunted by TGCI. $^{***}P < 0.001$, Student's unpaired t-test. (e) In contrast, the fractional change in astrocytic endfoot Fluo-4 fluorescence evoked by EFS was not different after TGCI ($n = 16$) compared to sham ($n = 14$). Here, 16 animals were used for each group, with lower n numbers reflecting attrition from unsuccessful preparations. (f) Similarly, the estimated endfoot baseline (sham: 108 ± 22 nM, n = 7; TGCI: 126 ± 30 nM, $n = 6$) and EFS-evoked (sham: 566 ± 140 nM, $n = 7$; TGCI: 626 ± 84 nM, $n = 6$) Ca^{2+} responses were unaffected by TGCI. (g) Typical images obtained during experiments in which bath K^+ was raised to 10 mM to stimulate K_{IR} channel-mediated dilations. While this produced robust dilation of arterioles in sham brain slices (top), in TGCI slices arteriolar diameter was unaffected (bottom). (h) Summary of 10 mM K^+-evoked increases in diameter. Peak dilations produced by this maneuver were significantly lower in slices from TGCI rats (13 ± 8 %, $n = 6$; compared to sham: 58 ± 9 %, $n = 6$). $^{**}P = 0.004$, Student's unpaired t-test.

increases in extracellular K^+ was severely compromised after ischemia. Consistent with the known role of myocyte K_{IR} channels in mediating dilation to extracellular K^+,[9,10,18] we observed a reduction in K_{IR} channel current density in PA myocytes from post-ischemic animals. Although we cannot exclude the contribution of other mechanisms to NVC impairment after TGCI, including altered release of other vasomodulatory substances from endfeet,[28–30] our findings strongly suggest a mechanism in which impairment of NVC after TGCI is caused by reduced myocyte K_{IR} channel function, disrupting K^+-mediated vasodilation.

Our observations support the concept that K_{IR} channel properties are plastic and are subject to modulation by pathological processes—such as cerebral ischemia/reperfusion[31,32]—which leads to alteration of NVC. K_{IR} channel activity may be suppressed by PKC and PIP_2,[33] both of which might be involved in ischemia and other cerebrovascular disorders.[34] In-depth investigation into how vascular myocyte K_{IR} channel

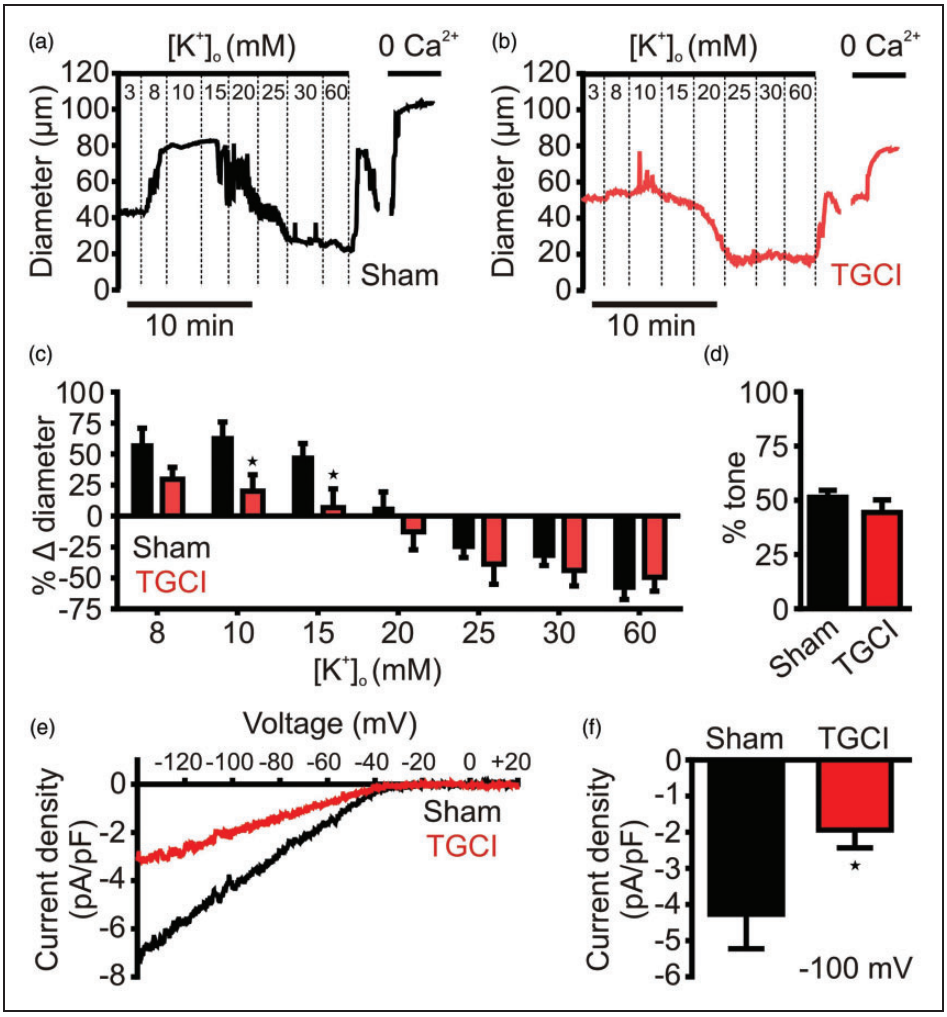

Figure 2. Parenchymal arteriole smooth muscle K_{IR} channel function is disrupted by TGCI. (a) Typical experimental time course showing diameter of an isolated pressurized (40 mm Hg) parenchymal arteriole from a sham rat to increasing concentrations of extracellular K^+. (b) Identical experiment to that displayed in panel A, for a parenchymal arteriole isolated from a TGCI rat. (c) Summary of dilations to increasing concentrations of K^+, dilation to 10 (sham: 72 ± 15%, $n = 7$; TGCI: 29 ± 15% $n = 8$) and 15 mM (sham: 51 ± 7%, n = 7; TGCI: 8 ± 12% $n = 8$) K^+ was significantly impaired in TGCI rats compared to controls, whereas constrictions were unaffected. *$P < 0.05$, one-way ANOVA with post hoc Bonferroni's multiple comparisons test. (d) Summary of myogenic tone induced by 40 mm Hg. Tone was no different between sham (51 ± 3%, $n = 7$) and TGCI (44 ± 6%, $n = 8$); 10 animals were used for each group, with lower n numbers reflecting attrition from unsuccessful experimental preparations. (e) Typical 100 μM Ba^{2+}-sensitive K_{IR} currents of parenchymal arteriole SMCs patch clamped in the whole-cell configuration with 60 mM external K^+, in response to a voltage ramp from −140 to +20 mV, showing a large inward current at potentials negative to E_K (−23 mV) and strong rectification at potentials depolarized to E_K. K_{IR} current density was greatly reduced in rats subjected to TGCI. (f) Summary data at −100 mV illustrating the significant reduction in K_{IR} current density in TGCI rat PA SMCs (−1.9 ± 0.5 pA/pF, $n = 9$) compared to sham controls (−4.3 ± 1.0 pA/pF, $n = 8$). *$P = 0.04$, Student's unpaired t-test.

function is disturbed after TGCI may reveal novel therapeutic approaches for restoring K_{IR} functionality and thereby normalizing NVC and local CBF after CA.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

GKP designed and carried out experiments, analyzed data and wrote and edited the paper. TAL assisted with experiments, analyzed data and wrote and edited the paper. AB performed electrophysiological experiments and analyzed data. DHE wrote and edited the paper. MTN coordinated overarching research design and wrote and edited the paper.

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