15-Oxo-ETE-induced internal carotid artery constriction in hypoxic rats is mediated by potassium channels

Short Title: 15-oxo-ETE induces ICA constriction in hypoxic rats

Di Wang, Yu Liu, Ping Lu, Daling Zhu, Yulan Zhu*

Department of Neurology, the Second Affiliated Hospital of Harbin Medical University

Address: 246 Xuefu Road, Harbin, Heilongjiang 150086, China

***Corresponding author:**
Yulan Zhu
Department of Neurology, the Second Affiliated Hospital of Harbin Medical University
246 Xuefu Road, Harbin, Heilongjiang 150086, China
Tel: 86-451-86297470, 86-18645049917
Fax: 86-451-86605656
E-mail: ylz0451@gmail.com

Summary

Our own study as well as others have previously reported that hypoxia activates 15-lipoxygenase (15-LO) in the brain, causing a series of chain reactions, which exacerbates ischemic stroke. 15-hydroxyeicosatetraenoic acid (15-HETE) and 15-oxo-eicosatetraenoic acid (15-oxo-ETE/15-KETE) are 15-LO-specific metabolites of arachidonic acid (AA). 15-HETE was found to be rapidly converted into 15-oxo-ETE by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in some circumstances. We have demonstrated that 15-HETE promotes cerebral vasoconstriction during hypoxia. However, the effect of 15-oxo-ETE upon the constriction of cerebral vasculature remains unclear. To investigate this effect and clarify the underlying mechanism, we performed Immunohistochemistry and Western blot to test the expression of 15-PGDH in rats' cerebral tissue, examined internal carotid artery (ICA) tension in isolated rat ICA rings. Western blot and reverse transcription polymerase chain reaction (RT-PCR) were used to analyze the expression of voltage-gated potassium (Kv) channels (Kv2.1, Kv1.5, and Kv1.1) in cultured cerebral arterial smooth muscle cells. The results showed that the levels of 15-PGDH expression were drastically elevated in the cerebral of rats with hypoxia, and 15-oxo-ETE enhanced ICA constriction in a dose-dependent manner. This effect was more significant in the hypoxic rats than in the normoxic rats. We also found that 15-oxo-ETE significantly attenuates the expression of Kv2.1 and Kv1.5, but not Kv1.1. In conclusion, these results suggest that 15-oxo-ETE leads to the contraction of the ICA, especially under hypoxic conditions and that specific Kv channels may play an important role in 15-oxo-ETE-induced ICA constriction.

Keywords: 15-oxo-eicosatetraenoic acid; 15-lipoxygenase; Voltage-gated potassium channels; Hypoxia; Cerebral vasoconstriction

Introduction

15-oxo-eicosatetraenoic acid (15-oxo-ETE/15-KETE) was originally shown to arise from 15-PGDH-mediated oxidation of 15-hydroxyeicosatetraenoic acid (15-HETE) in rabbit lung (Bergholte et al. 1987). 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) catalyzes the oxidation of 15(S)-hydroxyl group of prostaglandins and lipoxins, which has been considered the key enzyme responsible for the biological inactivation of these eicosanoids (Ensor and Tai 1995). 15-oxo-ETE was also observed as an arachidonic acid (AA) metabolite formed in human mast cells (Gulliksson et al. 2007). Previous studies have shown that the expression of 15-lipoxygenase (15-LO) in the internal carotid artery (ICA) was increased during hypoxia (Zhu et al. 2010). 15-LO catalyzes AA into 15-HETE and 15-oxo-ETE. 15-HETE was also found to be rapidly converted into 15-oxo-ETE (Guo et al. 2006, Wei et al. 2009). Our group demonstrated that 15-HETE can strengthen ICA vasoconstriction under hypoxic conditions (Zhu et al. 2010). 15-oxo-ETE has also been found to strengthen pulmonary artery constriction during chronic hypoxia (Guo et al. 2006). However, the role of 15-PGDH/15-oxo-ETE in hypoxia-induced ICA constriction and the underlying mechanism is still not clear.

There are four subtypes of potassium channels found in vascular smooth muscle cells, including voltage-gated potassium (Kv), ATP-sensitive K^+ (K_{ATP}), inward-rectifying potassium, and calcium-activated potassium (BK) channels (Chung et al. 2001). Hypoxia can inhibit Kv channels in most microcirculatory beds (Ward and Robertson 1995). Our previous studies have shown that 15-HETE induces ICA constriction through down-regulating Kv channels (Zhu et al. 2010), some of which (Kv1.5, Kv2.1, and Kv1.1) are sensitive to hypoxia (Amberg and Santana 2006, Gannushkina 2000). Because the mechanism of how 15-oxo-ETE enhances ICA constriction is unclear, it is necessary to demonstrate the relationship between 15-oxo-ETE and potassium channels under hypoxic conditions.

The present study was designed to elucidate the contraction properties of ICA rings induced by 15-oxo-ETE and investigate the underlying mechanisms of this reaction. The results show that the expression of 15-PGDH in internal carotid arteries

was examined during hypoxia, 15-oxo-ETE leads to constriction of the ICA, especially under hypoxic conditions, and 15-oxo-ETE-induced ICA constriction was dampened in the presence of a Kv channel inhibitor. Additionally, the protein and mRNA levels of Kv2.1 and Kv1.5 were also attenuated in cultured cerebral arterial smooth muscle cells (CASMCs) by the Kv channel inhibitor. Taken together, 15-HETE and 15-oxo-ETE may work together to promote hypoxia-induced cerebral vasoconstriction.

Materials and Methods

Materials

15-oxo-ETE was purchased from Cayman Chemical Company (Ann Arbor, USA). Antibody against 15-PGDH was purchased from Beijing Biosynthesis Biotechnology Co., LTD (Beijing, China). Potassium channel inhibitors 4-aminopyridine (4–AP), tetraethyl-ammonium (TEA) and glyburide (GLYB) were obtained from Sigma-Aldrich Co. (St. Louis, USA). Antibodies against Kv2.1, Kv1.5 and Kv1.1 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). The RT-PCR kit was acquired from Sangon Biotech Co. Ltd (Shanghai, China).

Animals and ICA preparation

Adult male Wistar rats with a mean weight of 200 g were obtained from the Experimental Animal Center of Harbin Medical University, which is fully accredited by the Institutional Animal Care and Use Committee (IACUC). The rats were raised in an environmental chamber, where the fractional inspired O_2 (FiO_2) was reduced to 12% (FIO_2 0.12) for nine days to establish hypoxic conditions. The rats raised in normoxic conditions (FIO_2 0.21) served as control (Zhu 2005). Normoxic rats were kept in the same room adjacent to the hypoxic chamber. Living temperature was 22 ± 2 °C, and relative humidity was 50 ± 10%. Food and water were given ad libitum. After nine days, we anesthetized each rat with pentobarbital (40 mg/kg, i.p.), and internal carotid arteries (ICA) were surgically removed for subsequent experiments.

The Immunohistochemistry of 15-PGDH

Rats were anesthetized (see above). 4% parafolmadehyde in 0.1 M phosphate buffer solution (PBS) was perfused into left ventricle/aorta until their bodies were

rigidity. The brains were quickly isolated and fixed in 4% parafolmadehyde PBS for additional 24 h. Cortical tissues were sliced in cross section at 20 μm by a freezing microtome. Sections were washed by PBS for three times and stained by 15-PGDH Immunohistochemistry (Zhu et al. 2005). The distribution of 15-PGDH (dark-brown in color) was observed under conventional optical microscope.

Tension studies of ICA rings

The arterial ring preparation was performed according to our previous reports (Zhu et al. 2010). Briefly, ICAs (1 - 1.5 mm in diameter) were isolated and cut into small segments (3 mm in length). The rings were bathed in pH-adjusted Krebs solution (mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; KH_2PO_4, 1.2; glucose, 6.0; pH 7.4) gassed with 5% CO_2 at 37 ℃. Tension of 0.3 g was incrementally applied over 30 min and then equilibrated for an additional 30 – 40 min at 37 ℃. Tension data were relayed from the pressure transducers to a signal amplifier (600 series eight-channel amplifier, Gould Electronics). Data were acquired and analyzed with CODAS software (Data Q Instruments, Inc). The following protocols were implemented after 1 h equilibration: Protocol 1 examined the relationships between vasoconstriction in ICA rings and 15-oxo-ETE dose, assessed in normoxic and hypoxic groups (n = 6). 15-oxo-ETE was added into Krebs solution at a concentration of 10^{-8} M to 10^{-6} M at 5 min intervals up to final concentration of 10^{-6} M. Protocol 2 examined the effects of 4-AP on responses to 15-oxo-ETE (10^{-8} - 10^{-6} M) in normoxic and hypoxic ICA rings. Protocol 3 examined the effects of TEA on responses to 15-oxo-ETE (10^{-8} - 10^{-6} M) in normoxic and hypoxic ICA rings. Protocol 4 examined the effects of GLYB on responses to 15-oxo-ETE (10^{-8} - 10^{-6} M) in normoxic and hypoxic ICA rings.

Culturing of rat CASMCs

The isolated ICA rings were cut into small pieces, dispersed in cultural medium with 4 mg/ml papain (Fu et al. 2004) for 18 min at 37 ℃, and transferred into the medium with collagenase (1 mg/ml, Invitrogen, USA) (Aoki et al. 2000) for 20 min at 37 ℃. The dispersed cells were centrifuged and resuspended in DMEM containing 20% fetal bovine serum (FBS) and 1% penicillin/streptomycin and then plated in T75

culture flasks in a humidified atmosphere of 5% CO_2 at 37 ℃ for three to five days. The purity of CASMCs in the primary cultures was confirmed by specific monoclonal antibodies raised against smooth muscle α-actin. Before each experiment, the cells were incubated in serum-free-DMEM for 24 h to stop cell growth, and CASMCs were divided into four groups. Group one (normoxic control) was maintained in an incubator with 5% CO_2 for 24h. Ethanol (1μmol/l) was added into the second group in an incubator containing 5% CO_2 for 24h as a vehicle control. The third group was incubated in a gas mixture composed of 3% O_2, 5% CO_2, and 92% N_2 for 24h. 1μmol/l 15-oxo-ETE was added to the fourth group in an incubator containing 5% CO_2 for 24h.

Western blot analysis

Proteins were extracted from CASMCs and brain tissue of rats, using the procedures essentially the same as described in detail elsewhere (Zhu et al. 2010). The protein concentration of each supernatant was determined using the Bradford method. An equal amount of protein (30 μg) from each sample was resolved in an 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membranes. After 1 h incubation in a blocking buffer containing 5% nonfat dry milk powder at room temperature, the blot was probed with specific antibodies against 29 kDa 15-PGDH (1:500), 96 kDa Kv2.1 (1:500), 67 kDa Kv1.5 (1:500), 56 kDa Kv1.1 (1:500), and 43 kDa β-actin (1:5,000) overnight at 4 ℃, followed by reaction with horseradish peroxidase-conjugated secondary antibodies, and a densitometric analysis was performed with an enhanced chemiluminescence detection system (Amersham, USA).

RT-PCR

The primers were designed according to the GenBank™ database as follows: Kv2.1 sense primer: 5’ – ACCATCGCTCTGTCACTCAA - 3’, anti-sense primer: 5’ - ACGCTCTTGTTGGATTCTGTG-3’, fragment size: 251 bp. Kv1.5 sense primer: 5’ - GGTCCTGGTCATTCTCATCTCT - 3’, anti-sense primer: 5’ - CACGAGCAACTCAAAAGTGAAC - 3’, fragment size: 256 bp. Kv1.1 sense primer: 5’ - AGCAGGAGGGGAATCAGAAG - 3’, anti-sense primer: 5’ -

ATCGGGGATACTGGAGAAGTG - 3', fragment size: 260 bp. β-actin sense primer: 5' – ACTATCGGCAATGAGCG - 3', anti-sense primer: 5' - GAGCCAGGGCAGTAATCT - 3', fragment size: 230 bp. Total RNA was extracted from cultured CASMCs by the acid guanidinium thiocyanate-phenol-chloroform extraction method and reverse-transcribed with the superscript first-strand cDNA synthesis kit. cDNA samples were amplified in a DNA thermocycler (PerkinElmer), and the PCR products were visualized by ethidium bromide-stained agarose gel electrophoresis. β-actin mRNA was used as an internal control to quantify PCR products. The OD values in the channel signals, measured by a Kodak electrophoresis documentation system, were normalized to those of β-actin. The ratios are expressed as arbitrary units for quantitative comparison.

Statistical analysis

The experimental data are expressed as the mean ± SD. Statistical analysis was made using one-way analysis of variance (ANOVA) followed by appropriate Dunnett's test. Differences were considered to be significant if $p \leq 0.05$.

Results

The expression of 15-PGDH in ICA of rats with hypoxia

The expression of 15-PGDH in internal carotid arteries (ICA) was examined during hypoxia. Two groups of rats were placed under the conditions of hypoxia and control, respectively (see Methods and materials section). Nine days after the treatments, rats' brain tissue was isolated for Immunohistochemistry. As shown in Fig. 1A, the levels of 15-PGDH were higher in hypoxia than in normoxia, indicating that hypoxia elevates 15-PGDH expression. To further confirm that 15-PGDH expression is elevated in hypoxia ICA, we analyzed ICAs from rats by Western blot. 15-PGDH protein was strongly induced in rats with hypoxia (Fig. 1C, D).

Effects of 15-oxo-ETE on normoxic and hypoxic ICA rings

Isometric ICA contractions were studied in normoxic and hypoxic rings. Administration of exogenous 15-oxo-ETE led to slow constrictions of both normoxic and hypoxic rings across a range of concentrations. This effect was greater in hypoxic rings than in normoxic rings and was readily apparent using concentrations of 10^{-6}

and 10^{-7} mol/l 15-oxo-ETE, suggesting that 15-oxo-ETE enhances the sensitivity of ICA to hypoxia (Fig. 2A, 2B). Because 15-oxo-ETE was diluted with ethanol, we ran a vehicle group to control for effects of the solvent. The results showed that the solvent had no effect on the contraction of ICA rings.

Effects of 4-AP, TEA, and GLYB on 15-oxo-ETE-induced ICA constriction

To determine which type of potassium channels were involved in the constriction of hypoxic ICA rings, the Kv channel blocker 4-aminopyridine (4-AP), the BK channel blocker tetraethyl-ammonium (TEA), and the K_{ATP} channel blocker glyburide (GLYB) were used in rat ICA tension studies. ICA rings isolated from normoxic and hypoxic rats were incubated with 2 mmol/L 4-AP, 10^{-2} mmol/l TEA and 10^{-6} mmol/l GLYB for 30 min. Untreated ICA rings were used as a control. A series of concentration response curves were produced when the ICA rings were treated with incremental concentrations of 15-oxo-ETE in the range from 10^{-8} to 10^{-6} M. As shown in Fig. 3, pretreatment of rat ICA rings with 4-AP significantly diminished constriction of the rings after adding different concentrations of 15-oxo-ETE. However, constriction of the rings in the TEA and GLYB treated groups was not significantly attenuated. In nearly every case, greater constriction was observed compared with the untreated control (Fig. 4). These results indicate that 15-oxo-ETE-induced ICA constriction during hypoxia may be mediated by the inhibition of Kv channels.

Effects of 15-oxo-ETE on Kv channels

We then examined whether 15-oxo-ETE reduced the expression of Kv channels in CASMCs using Western blot and RT-PCR. As seen in Fig. 5A, western-blot data shows that the protein expression of Kv2.1 are lighter under both hypoxic conditions and 15-oxo-ETE treatment than normoxia and vehicle (1 μmol/l ethanol was added into the CASMCs as solvent control group) respectively. Fig. 5B illustrates the relative values of Kv2.1 under the conditions of normoxia, vehicle treatment, hypoxia, and 15-oxo-ETE treatment. As seen in Fig. 5B, the levels of Kv2.1 protein are significantly lower under hypoxia and 15-oxo-ETE than normoxia and vehicle ($p < 0.05$, n = 6). RT-PCR data show that the mRNA expression of Kv2.1 is lighter under

hypoxia and 15-oxo-ETE than normoxia and vehicle (Fig. 5C). The relative values of Kv2.1 under the conditions of normoxia, vehicle, hypoxia, and 15-oxo-ETE are shown in Fig. 5D. The levels of Kv2.1 mRNA are significantly lower under hypoxia and 15-oxo-ETE than normoxia and vehicle.

Fig. 6 shows the effects of 15-oxo-ETE and hypoxia on Kv1.5 expression. In western-blot and RT-PCR analysis of Kv1.5 protein and mRNA, the protein or mRNA expression of Kv1.5 are lighter under hypoxia and 15-oxo-ETE than normoxia and vehicle (Fig. 6A, C). The relative values of Kv1.5 under the conditions of normoxia, vehicle, hypoxia and 15-oxo-ETE are shown in Fig. 6B and Fig. 6D.

We further examined whether Kv1.1 was involved in the 15-oxo-ETE-induced suppressive effects, finding that there was no significant change in protein and mRNA expression.

Discussion

This study provides novel evidence that Kv channels are involved in 15-oxo-ETE-induced ICA constriction. In our previous studies, we found that hypoxic exposure induces the expression of 15-LO in CASMCs (Zhu et al. 2010). 15-LO catalyzes the production of 15-HETE and 15-oxo-ETE (Wei et al. 2009), and 15-HETE is an important mediator in regulating the cerebral vasoconstriction, as its primary target is the vascular smooth muscle. In this study, we found that the level of 15-PGDH, which converts arachidonic acid into 15-oxo-ETE in ICA is higher in hypoxia than control (Fig. 1). 15-oxo-ETE-induced vasoconstriction is mediated by down-regulation of potassium channels. This is a new mechanism that explains why hypoxia intensifies cerebral vasoconstriction, exacerbating cerebral ischemia.

Wei et al. reported that 15-oxo-ETE was found to be a major AA-derived LO metabolite when AA was given exogenously (Wei et al. 2009). Hypoxia elevates 15-LO expression in the brain (Zhu et al. 2005) and 15-oxo-ETE has been found to strengthen pulmonary artery constriction (Guo et al. 2006). Our study demonstrates that 15-oxo-ETE induces dose-dependent ICA constriction, a result much more apparent under hypoxia than normoxia (Fig. 2). Thus, we propose that a chain reaction mechanism occurs during hypoxia, in which the over-expressed 15-PGDH

converts AA into 15-oxo-ETE, and 15-oxo-ETE strengthens ICA vasoconstriction to aggravate vascular occlusion during brain ischemia. Our research brings new insight into the pathology of cerebral ischemia.

It is well-known that potassium channels have extensive biological activity, and several types of potassium channels have been validated, including voltage-gated potassium, ATP-sensitive K^+, inward rectification, and calcium-activated potassium channels (Chung et al. 2001). A range of experimental approaches has revealed that voltage-gated potassium (Kv) channels increase their activity with membrane depolarization and are important regulators of smooth muscle membrane potential in response to depolarizing stimuli (Nelson and Quayle 1995).
BK channels exhibit various functions such as action potential repolarization, blood pressure regulation, hormone secretion, and transmitter release (Handlechner et al. 2013) and are overexpressed in human gliomas and brain tumor endothelial cells (Khaitan and Ningaraj 2013). ATP-sensitive potassium (K_{ATP}) channels play a key role in insulin secretion by coupling metabolic signals to β-cell membrane potential (Chen et al. 2013). However, which types of potassium channels are responsible for 15-oxo-ETE-induced hypoxic cerebral vasoconstriction? In our study, we used inhibitors of these types of potassium channels in rat ICA tension studies to clarify the question. Administration of the Kv channel blocker 4-AP attenuates the effect of 15-oxo-ETE on ICA constriction (Fig. 3), which suggests that 15-oxo-ETE's contraction effect on ICA may be related to voltage-gated potassium channels.

The Kv channels comprise a large family with many subtypes. A study has demonstrated that Kv2.1 is an important regulator of resting membrane potential in pulmonary artery smooth muscle cells (Archer et al. 1998). Another report showed that voltage-gated potassium channel Kv3.3 is the causative gene of spinocerebellar ataxia type 13 (Zhao et al. 2013). Liebau et al. demonstrated that pharmacological inhibition of Kv1.3 increased neural progenitor cell proliferation (Liebau et al. 2006). Some Kv channels (Kv2.1, Kv1.5, Kv1.1) are sensitive to hypoxia (Amberg and Santana 2006, Gannushkina 2000). A substantial component of the voltage-dependence and kinetics of Kv currents in cerebral arterial myocytes is

mediated by Kv1 and Kv2 channels (Amberg and Santana 2006). Thus, we investigated the expression of Kv 2.1, Kv1.5 and Kv1.1. Our data show that hypoxia and 15-oxo-ETE attenuate the expression of Kv2.1 (Fig. 5) and Kv1.5 (Fig. 6), but not Kv1.1. Here we demonstrate for the first time that Kv2.1 and Kv1.5 channels may play a critical role in 15-oxo-ETE-induced ICA vasoconstriction during hypoxia.

Based on our study, 15-oxo-ETE produced during hypoxia down-regulates Kv1.5 and Kv2.1 expression, and inhibition of Kv channels prolongs spike repolarization and lowers resting membrane potentials. The prolonged spike repolarization allows a dominant Ca^{2+} influx in CASMCs during action potentials, lowering resting potentials and reducing the energetic barrier to fire action potentials. All the above may elevate cytoplasmic Ca^{2+} and in turn induce cerebral vasoconstriction during hypoxia and ischemia. It remains to be seen how Ca^{2+} channels and Ca^{2+} influx are involved in hypoxic vasoconstriction, a subject of our future studies.

In conclusion, the present study suggests that 15-oxo-ETE-induced ICA constriction in hypoxic rats is mediated by specific types of voltage-gated potassium channels. 15-oxo-ETE is a promoter of hypoxic ICA constriction, and Kv2.1/1.5 channels may be the targets of 15-oxo-ETE. In addition, 15-oxo-ETE and 15-HETE may work together to regulate hypoxic cerebral vasoconstriction. These findings may not only help in understanding the mechanisms of 15-oxo-ETE action in hypoxic cerebral vasoconstriction but also in pointing out a new therapeutic target for cerebral ischemic disorders.

Acknowledgments

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References

Amberg GC, Santana LF: Kv2 channels oppose myogenic constriction of rat cerebral arteries. Am J Physiol Cell Physiol **291**: C348-C356, 2006.
Aoki K, Zubkov AY, Parent AD, Zhang JH: Mechanism of ATP-induced [Ca2+] i mobilization in rat basilar smooth muscle cells. Stroke **31**:1377-1385, 2000.
Archer SL, Souil E, Dinh-Xuan AT, Schremmer B, Mercier J-C, El Yaagoubi A: Molecular identification of the role of voltage-gated K+ channels, Kv1. 5 and Kv2. 1, in hypoxic pulmonary vasoconstriction and control of resting membrane potential in rat pulmonary artery myocytes. J Clin Invest **101**: 2319, 1998.
Bergholte JM, Soberman RJ, Hayes R, Murphy RC, Okita RT: Oxidation of 15-hydroxyeicosatetraenoic acid and other hydroxy fatty acids by lung prostaglandin dehydrogenase. Arch Biochem Biophys **257**: 444-450, 1987.
Chen PC, Olson EM, Zhou Q, Kryukova Y, Sampson HM, Thomas DY: Carbamazepine as a Novel Small Molecule Corrector of Trafficking-impaired ATP-sensitive Potassium Channels Identified in Congenital Hyperinsulinism. J Biol Chem **288**: 20942-20954, 2013.
Chung YH, Kim HS, Shin CM, Kim MJ, Cha CI: Immunohistochemical study on the distribution of voltage-gated K(+) channels in rat brain following transient focal ischemia. Neurosci Lett **308**: 157-160, 2001.
Ensor CM, Tai HH: 15-Hydroxyprostaglandin dehydrogenase. J. Lipid Mediat. Cell Signal. **12**: 313 – 319, 1995.
Fu ZJ, Xie MJ, Zhang LF, Cheng HW, Ma J: Differential activation of potassium channels in cerebral and hindquarter arteries of rats during simulated microgravity. Am J Physiol - Heart C **287**: H1505-H1515, 2004.
Guo S, Zhang Y, Liu Y: Effect of 15-KETE on rat isolated pulmonary rings. Chin Pharmacol Bull **22**: 1339-1343, 2006.
Gannushkina IV: Cerebral circulation in different types of brain hypoxia. Vestn Ross Akad Med Nauk: 22-27, 2000.
Gulliksson M, Brunnström Å, Johannesson M, Backman L, Nilsson G, Harvima I: Expression of 15-lipoxygenase type-1 in human mast cells. BBA-Mol Cell Biol L **1771**: 1156-1165, 2007.
Handlechner AG, Hermann A, Fuchs R, Weiger TM: Acetaldehyde-ethanol interactions on calcium-activated potassium (BK) channels in pituitary tumor (GH3) cells. Front Behav Neurosci **7**: 58, 2013.
Khaitan D, Ningaraj NS: Targeting potassium channels for increasing delivery of imaging agents and therapeutics to brain tumors. Front Pharmacol **4**: 62, 2013.
Liebau S, Pröpper C, Böckers T, Lehmann‐Horn F, Storch A, Grissmer S: Selective blockage of Kv1. 3 and Kv3. 1 channels increases neural progenitor cell proliferation. J Neurochem **99**: 426-437, 2006.
Nelson MT, Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol - Cell Ph **268**: C799-C822, 1995.
Ward JP, Robertson TP: The role of the endothelium in hypoxic pulmonary vasoconstriction. Exp Physiol **80**: 793-801, 1995.
Wei C, Zhu P, Shah SJ, Blair IA: 15-oxo-Eicosatetraenoic acid, a metabolite of macrophage 15-hydroxyprostaglandin dehydrogenase that inhibits endothelial cell

proliferation. Mol Pharmacol **76**: 516-525, 2009.
Zhao J, Zhu J, Thornhill WB: Spinocerebellar ataxia-13 Kv3.3 potassium channels: arginine-to-histidine mutations affect both functional and protein expression on the cell surface. Biochem J **454**: 259-265, 2013.
Zhu Y, Chen L, Liu W, Wang W, Zhu D, Zhu Y: Hypoxia-induced 15-HETE enhances the constriction of internal carotid arteries by down-regulating potassium channels. J Neurol Sci **295**: 92-96, 2010.
Zhu Y, Shan H, li Q, Zhu D: Contractile effect and mechanism of 15-HETE on hypoxic rat ICA rings. Chin Pharmacol Bull **21**:1327-1332, 2005.

Figure legends

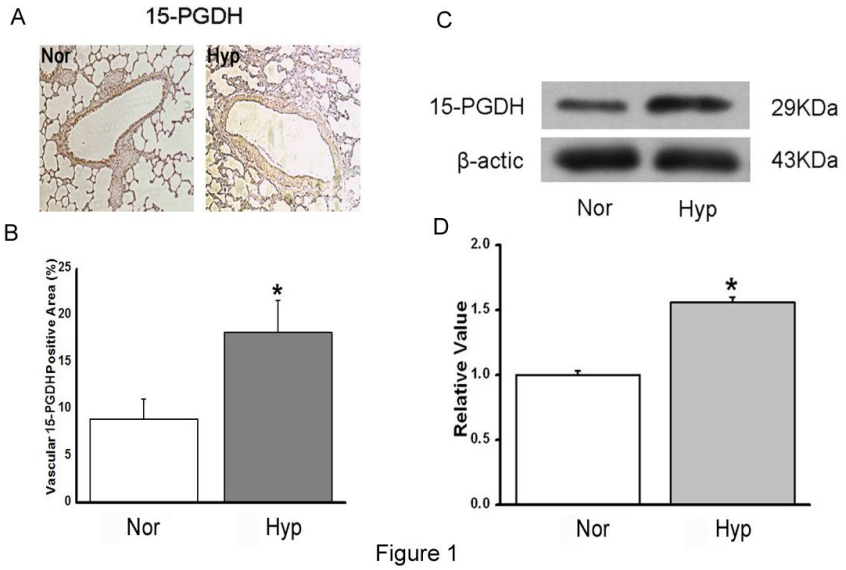

Figure 1

15-Hydroxyprostaglandin dehydrogenase (15-PGDH) expression is increased in hypoxic rats. 'Nor' refers to rats under normoxic conditions, 'Hyp' refers to rats under hypoxic conditions. (A) 15-PGDH expression in smooth muscle cells of ICAs from rats was evaluated by Immunohistochemistry. (B) The quantitative data for vascular 15-PGDH positive area. (C) The protein expression of 15-PGDH in ICAs from normoxic and hypoxic rats was detected by Western blot. (D) The quantitative data for 15-PGDH proteins. (n = 6, * indicates $p < 0.05$)

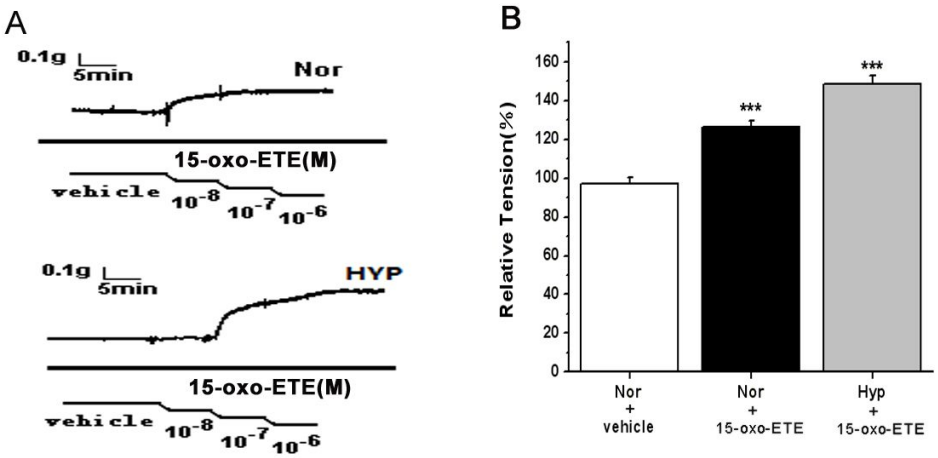

Figure 2

Figure 2

Constrictive effects of 15-oxo-ETE on internal carotid arteries (ICAs) under normoxic and hypoxic conditions. 'Nor' refers to rats under normoxic conditions, 'Hyp' refers to rats under hypoxic conditions. 'vehicle' is the solvent control group. (A) Concentration response curves from the signal amplifier (600 series eight-channel amplifier, Gould Electronics). (B) The quantitative data for 15-oxo-ETE-induced ICA constriction. (n = 6, *** indicates $p < 0.001$)

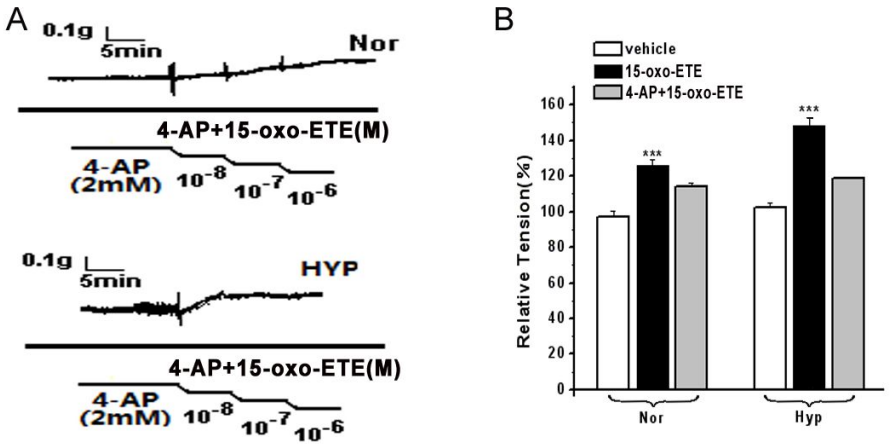

Figure 3

Figure 3

Effects of 4-AP on 15-oxo-ETE-induced ICA constriction. (A) Concentration

response curves from the signal amplifier (600 series eight-channel amplifier, Gould Electronics). (B) The quantitative data for 4-AP on 15-oxo-ETE-induced ICA constriction under normoxic and hypoxic condition. (n = 6, *** indicates $p < 0.001$)

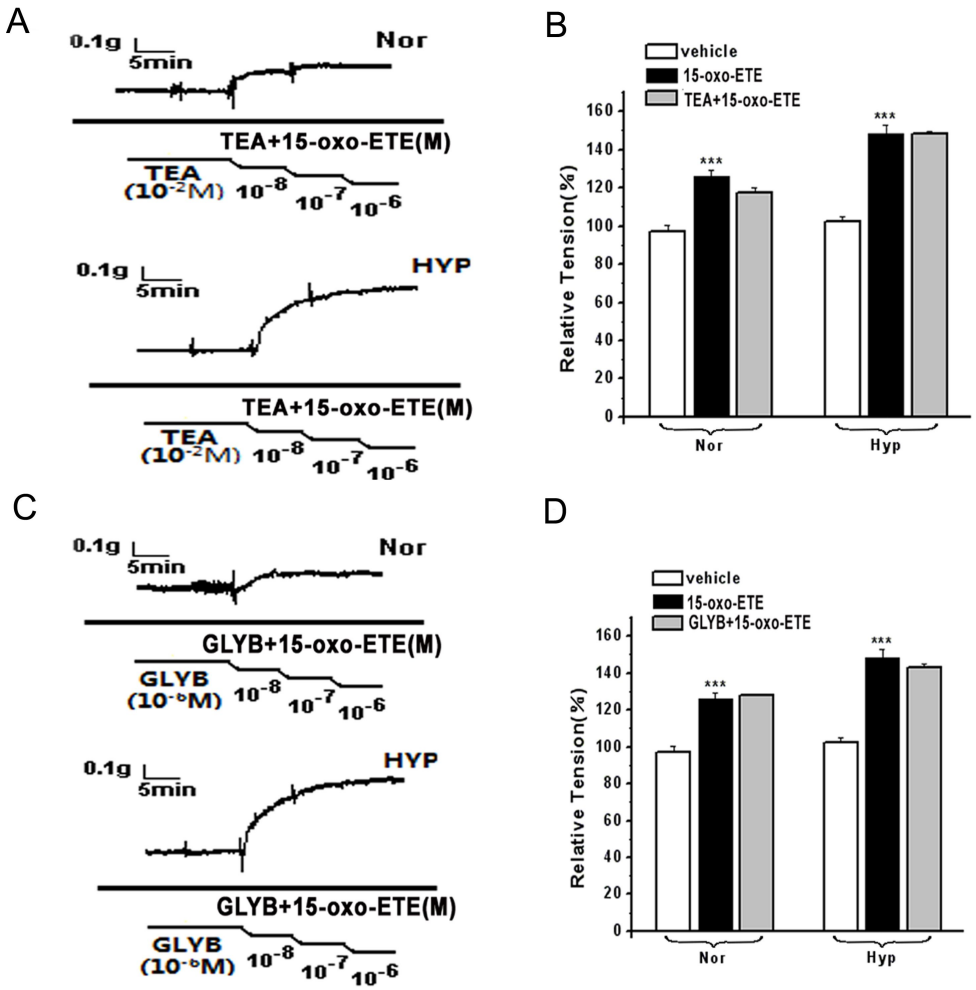

Figure 4

Figure 4

Effects of TEA and GLYB on 15-oxo-ETE-induced ICA constriction. (A) Concentration response curves from the signal amplifier (600 series eight-channel amplifier, Gould Electronics). (B) The quantitative data for TEA on 15-oxo-ETE-induced ICA constriction under normoxic and hypoxic condition. (C) Concentration response curves from the signal amplifier (600 series eight-channel amplifier, Gould Electronics). (D) The quantitative data for GLYB on 15-oxo-ETE-induced ICA constriction under normoxic and hypoxic condition. (n = 6, *** indicates $p < 0.001$)

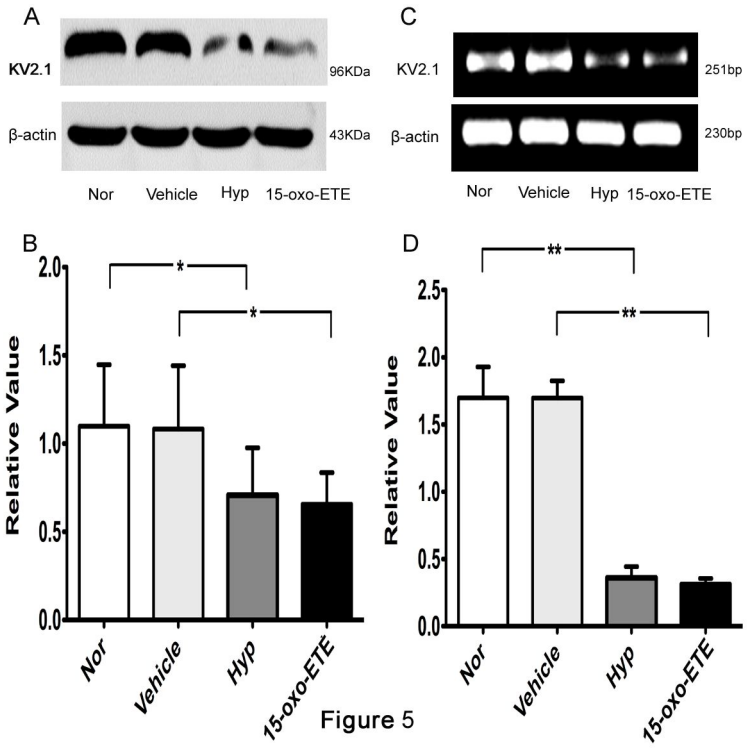

Figure 5

Hypoxia and 15-oxo-ETE attenuate the levels of Kv2.1 in smooth muscle of internal carotid arteries (ICA). (A) The levels of Kv2.1 protein (Pr) detected by Western blot under the conditions of normoxia, vehicle, hypoxia and 15-oxo-ETE. (B) The quantitative data for Kv2.1 proteins. (C) The levels of Kv2.1 mRNA detected by RT-PCR under the conditions of normoxia, vehicle, hypoxia and 15-oxo-ETE. (D) The quantitative data for Kv2.1 mRNAs. (n = 6, * indicates $p < 0.05$, **indicates $p < 0.01$)

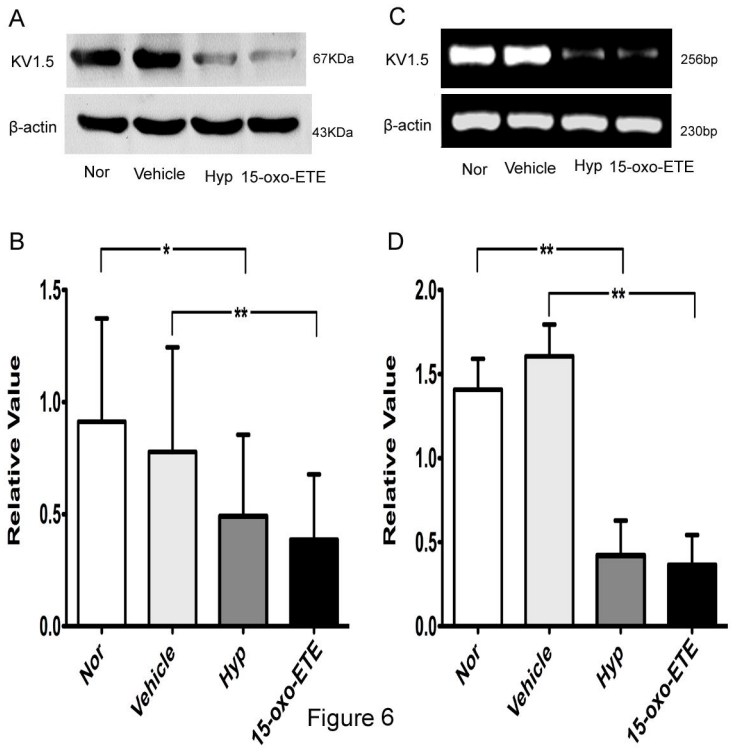

Figure 6

Hypoxia and 15-oxo-ETE down-regulate the levels of Kv1.5 in smooth muscle of internal carotid arteries (ICA). (A) The levels of Kv1.5 protein (Pr) detected by Western blot under the conditions of normoxia, vehicle, hypoxia and 15-oxo-ETE. (B) The quantitative data for Kv1.5 proteins. (C) The levels of Kv1.5 mRNA detected by RT-PCR under the conditions of normoxia, vehicle, hypoxia and 15-oxo-ETE. (D) The quantitative data for Kv1.5 mRNAs. (n = 6, * indicates $p < 0.05$, **indicates $p < 0.01$)