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Effects of EP1 Receptor on Cerebral Blood Flow in the Middle Cerebral Artery Occlusion Model of Stroke in Mice

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Abstract

The lipid mediator prostaglandin E₂ (PGE₂) exhibits diverse biologic activity in a variety of tissues. Four PGE₂ receptor subtypes (EP1–4) are involved in various physiologic and pathophysiologic conditions, but differ in tissue distribution, ligand-binding affinity, and coupling to intracellular signaling pathways. To characterize the role of the EP1 receptor, physiologic parameters (mean arterial blood pressure, pH, blood gases PaO₂ and PaCO₂, and body temperature), cerebral blood flow (CBF), and neuronal cell death were studied in a middle cerebral artery occlusion model of ischemic stroke in wild-type (WT) and EP1 knockout (EP1^{−/−}) mice. The right middle cerebral artery was occluded for 60 min, and absolute CBF was measured by [¹⁴C] iodoantipyrine autoradiography. The effect of EP1 receptor on oxidative stress in neuronal cultures was investigated. Although no differences were observed in the physiologic parameters, CBF was significantly ($P < 0.01$) higher in EP1^{−/−} mice than in WT mice, suggesting a role for this receptor in physiologic and pathophysiologic control of vascular tone. Similarly, neuronal cultures derived from EP1^{−/−} mice were more resistant ($90.6 \pm 5.8\%$ viability) to *tert*-butyl hydroperoxide-induced oxidative stress than neurons from WT mice ($39.6 \pm 17.2\%$ viability). The EP1 receptor antagonist SC-51089 and calcium channel blocker verapamil each attenuated the neuronal cell death induced by PGE₂. Thus, the prostanoid EP1 receptor plays a significant role in regulating CBF and neuronal cell death. These findings suggest that pharmacologic modulation of the EP1 receptor might be a means to improve CBF and neuronal survival during ischemic stroke.

Keywords

brain ischemia; oxidative stress; neuroprotection; prostanoid; SC-51089

The goal in the management of brain injury is to minimize the consequences of secondary injury. It is best to manage the primary injury that occurs at the time of event (trauma or cerebrovascular insult) to prevent secondary injury by controlling the abnormal physiology encountered after brain injury. Ischemic stroke activates an inflammatory reaction in the injured brain that advances for days after the onset of symptoms. Some evidence suggests that such inflammatory reactions contribute to the late stages of ischemic injury and worsen neurologic outcome (Doré et al., 2003).

Prostanoids are endogenous signaling molecules produced locally at their site of action via the enzymatic transformation of arachidonic acid by cyclooxygenase (COX) isoenzymes. Five

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naturally occurring prostanoids, namely prostaglandins PGD₂, PGE₂, PGF_{2α}, PGI₂, and thromboxane A₂, have been identified together with corresponding receptor families through which they mediate their actions (Narumiya et al., 1999; Narumiya and FitzGerald, 2001). Prostanoids such as PGE₂ are produced during inflammatory responses, and increased levels of PGE₂ help to mediate inflammatory processes, such as those involved with cerebral ischemia (Doré et al., 2003), spinal trauma (Nakayama et al., 2004), edema (Guay et al., 2004), and fever (Oka, 2004).

PGE₂ mediates its physiologic effects by interacting with a subfamily of G-protein-coupled receptors known as EP receptors (Narumiya et al., 1999). Stimulation of the EP1 receptor results in activation of phosphatidyl inositol hydrolysis and elevation of the intracellular Ca²⁺ concentration. EP2 and EP4 activation increase intracellular cAMP, whereas EP3 activation decreases it (Audoly et al., 1999). PGE₂ has been implicated as an endogenous modulator of both vasoconstriction and vasorelaxation in several species, depending on which receptor it binds (Li et al., 1996). For example, EP1 and EP3 receptors mediate PGE₂-induced constriction in porcine large cerebral arteries (Jadhav et al., 2004) and in human pulmonary arteries (Norel et al., 2004), whereas EP4 receptors mediate vasodilatation of human middle cerebral arteries (Davis et al., 2004). In addition, EP1 receptors mediate pain perception, regulate blood pressure (Stock et al., 2001), and are involved in control of vascular tone (Zonta et al., 2003a,b) and development of renal injury in genetically hypertensive rats (Suganami et al., 2003).

Although selective COX-2 inhibitors have been used to treat such conditions as inflammation and pain, recent reports have shown that those drugs pose a hazard of myocardial infarction and stroke (Wong et al., 2005; Rocca, 2006). Defining the prostaglandin pathways associated with disease attenuation and promotion would provide useful targets for new, more specific therapeutic strategies. We chose to focus on the EP1 receptor (Funk et al., 1993) because in vitro studies have shown a dual role of PGE₂ in excitotoxicity via activation of the EP1 receptor and preconditioning-induced neuroprotection (Gendron et al., 2005). Similarly, our previous results and other studies have shown that the deletion of the EP1 receptor was protective in models of excitotoxicity and focal ischemic brain damage (Ahmad et al., 2006; Kawano et al., 2006). During ischemia, the EP1 receptor might alter cerebral blood flow (CBF) or affect neuronal cells directly. To determine which mechanism is active during stroke, we measured the CBF during and after middle cerebral artery (MCA) occlusion (MCAO) in wild-type (WT) and EP1 knockout (EP1^{-/-}) mice and neuronal cell death in cortical neurons derived from WT and EP1^{-/-} mice.

MATERIALS AND METHODS

Animals

This study was conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals. The animal protocols used for these studies were approved by the Institutional Animal Care and Use Committee at Johns Hopkins University. Adult male WT and EP1^{-/-} C57BL/6 mice were generously provided by Shuh Narumiya (University of Kyoto, Japan).

MCAO and Reperfusion

Transient focal cerebral ischemia was induced by occluding the MCA with an intraluminal filament. Under halothane anesthesia (3.0% for induction, 1.0% for maintenance), adult male mice (20–28 g at 8–10 weeks old) were ventilated with oxygen-enriched air via a nose cone. Body temperatures were maintained at 37.0 ± 0.5°C by a heating pad. Relative CBF was measured by laser-Doppler flowmetry (Moor Instruments, Devon, England) with a flexible

probe affixed to the skull over the parietal cortex supplied by the MCA (2 mm posterior and 6 mm lateral to the bregma). The neck and carotid bifurcation were dissected under aseptic conditions. The common carotid artery was ligated temporarily, and the external carotid artery was used as a stump. A small 7–0 Ethilon nylon monofilament (Ethicon, Somerville, NJ) covered with flexible silicone (CutterSil Light, Heraeus Kulzer GmbH, Hanau, Germany) and Universal hardener (CutterSil, Heraeus Kulzer Dormagen, Germany) was inserted into an incision in the external carotid artery stump and advanced through the internal carotid artery to the origin of the MCA. Correct placement was documented by a decrease in laser-Doppler signal. Anesthesia was discontinued while the filament tip was left in position for 90 min. After 90 min of occlusion, the mice were briefly anesthetized with halothane, and reperfusion was achieved by withdrawing the filament and reopening the MCA, after which, the neck was sutured. The animals were allowed to survive for 1 hr after reperfusion.

Regional CBF Assessment

In a separate cohort of mice, CBF was measured at endischemia by [^{14}C] iodoantipyrine ([^{14}C]-IAP) autoradiography (Jay et al., 1988), as described previously in mouse (Sawada et al., 2000). Four WT and four EP1 $^{-/-}$ mice were anesthetized with halothane, and the right femoral arteries and veins were catheterized. At 60 min of ischemia, 4 μCi of [^{14}C]-IAP were infused intravenously at a constant rate of 108 $\mu\text{l}/\text{min}$ for 45 sec. Arterial blood was sampled at 5-sec intervals to obtain the arterial input function as described (Sampei et al., 2000). The total volume of blood withdrawn was 100–160 μl . At 45 sec of infusion, the anesthetized mouse was decapitated, and the brain was quickly removed, frozen in 2-methylbutane on dry ice, and stored at -80°C . Later, the brain was sliced into 20- μm -thick coronal sections on a cryostat, thaw-mounted onto glass cover slips, and apposed to Kodak SB-5 film (Eastern Kodak Company, Rochester, NY) for 1 week with ^{14}C standards. Nine autoradiographic images at each of six coronal levels (+2, +1, 0, -1, -2, -3 mm from the bregma) were digitized, and regional blood flow was calculated with image analysis software (Inquiry, Loats Associates, Westminster, MD).

Measurement of Body Temperature, Blood Gases, and Mean Arterial Blood Pressure

Body temperature was determined with a rectal probe in separate cohorts of mice ($n = 5$) at baseline, at 15-min intervals during 90 min of ischemia, and for 60 min of reperfusion. The femoral artery was cannulated for measurement of arterial blood gases (pH, PaO_2 , and PaCO_2) at baseline, 60 min of occlusion, and 60 min after reperfusion. Mean arterial blood pressure (MABP), also measured via the femoral catheter, was recorded at baseline and at 15-min intervals during ischemia and reperfusion.

Measurement of Cerebral Vessel Diameter

To examine the anatomy of the large vessels in WT and EP1 $^{-/-}$ mice, three naive mice of each genotype were deeply anesthetized and perfused via the heart left ventricle with 5 ml of ice-cold saline followed by 1 ml of black latex paint. Then the mice were decapitated, and their brains removed with the circle of Willis intact. Brains were immersed in 10% formalin for 24 hr before examination. The vessel diameter was measured with Metavue software (Meta Imaging Series Software, Downingtown, PA).

Cell Culture

Cortical neurons were cultured from 1–2-day-old postnatal WT and EP1 $^{-/-}$ mice as described previously (Echeverria et al., 2005). Briefly, the brains were harvested from the neonatal mice and dissected under a microscope to remove the blood vessels and meninges. The cortices were then dissociated by 0.05% (v/v) trypsin digestion and triturated by repeated passages through a pipette tip. Cells (5×10^5) were plated at high-density (2,500 cells/ mm^2) in neurobasal

medium supplemented with 1 mM Glutamax and B27 (Gibco, Grand Island, NY) onto 24-well plates precoated with poly-D-lysine (Sigma, St. Louis, MO). The cortical cell cultures were kept at 37°C in a humidified incubator with 95% air/5% CO₂ until used. All experiments were carried out at 10–14 days in vitro.

Measurement of Cell Survival

Cortical neurons derived from WT and EP1^{-/-} mice were exposed to freshly prepared *tert*-butyl hydroperoxide (*t*-BuOOH, an inducer of oxidative stress); PGE₂; the calcium channel blocker verapamil; the EP1 receptor antagonist SC-51089 (8-chlorodibenz[*b,f*][1,4]oxazepine-10(11*H*)-carboxylic acid; 2-[1-oxo-3-(3-pyridinyl)propyl]hydrazide); or a combination for 24 hr. Neuronal survival was quantified with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Sigma), an indicator of the mitochondrial activity of living cells, or the lactate dehydrogenase (LDH) assay (Sigma), an indicator of the LDH leakage into the culture medium. For the MTT assay, cells were incubated for 2 hr at 37°C with 0.5 mg/ml MTT, and living cells containing MTT formazan crystals were solubilized in a solution of anhydrous isopropanol, 0.1 N HCl, and 0.1% Triton X-100. The optical density was determined at 570 nm. For the LDH assay, culture medium was transferred to a clean flat-bottom plate, and the assay substrate was added in an amount equal to 50% of the volume of culture medium. Samples were incubated at room temperature for 30 min in the dark. The reaction was terminated by the addition of 1/10 volume 1 N HCl to each well. Absorbance was measured at a wavelength of 490 nm with a multiplate reader, POLARstar OPTIMA (BMG LABTECH, Durham, NC). Experiments were repeated with at least three separate batches of cultures.

Statistical Analysis

Data are expressed as means ± SE. Multiple comparisons were analyzed by the ANOVA and Tukey's test, with significance set at $P < 0.05$.

RESULTS

First, we investigated whether there were anatomic differences in the large cerebral vessels that might affect cerebral blood flow, but with our experimental protocol, we found no significant differences in the vessel diameters of WT and EP1^{-/-} mice (Fig. 1).

Table I shows physiologic measurements at baseline, during ischemia, and after 1 hr of reperfusion ($n = 5$ per genotype). PaO₂ was significantly lower ($P < 0.05$) in EP1^{-/-} mice than in WT mice during MCAO, but no differences were noted in pH or PaCO₂ at any time point.

CBF was measured before MCAO and every 15 min during occlusion and reperfusion. During occlusion, the CBF decreased by more than 80% in the WT and EP1^{-/-} mice; no significant difference between the two groups was observed (Fig. 2). However, during reperfusion, CBF recovered to a significantly higher level in EP1^{-/-} mice than in WT mice ($P < 0.05$). During the length of the study, no significant changes in body temperature were observed in either group (Fig. 3). In addition, with the mice exposed to equal levels of anesthesia, MABP decreased similarly in both mouse groups during MCAO and reperfusion (Fig. 4).

To determine the role of EP1 in regulation of CBF, we calculated CBF using quantitative [¹⁴C]-IAP autoradiography. Potential differences between EP1^{-/-} and WT mice in vascular responsiveness were examined by quantifying absolute regional CBF in the parietal cortex, lateral cortex, and ventrolateral and dorsomedial caudate putamen in the ischemic and contralateral, nonischemic hemispheres. After 60 min of occlusion, the ipsilateral CBF was significantly lower in the lateral cortex, ventrolateral and dorsomedial caudate putamen ($P <$

0.01) and in the parietal cortex 1 ($P < 0.05$) of WT mice than of EP1^{-/-} mice (Fig. 5). However, there was no difference in CBF in the parietal cortex 2 between the two groups of mice. CBF in the nonischemic contralateral hemisphere also was similar in EP1^{-/-} and WT mice.

Considering that we and others have shown that the infarct size after ischemia-reperfusion in EP1^{-/-} mice is attenuated compared to that of WT controls (Ahmad et al., 2006; Kawano et al., 2006), here we addressed the potential direct effect of EP1 on neuronal survival. We used postnatal cortical neurons derived from EP1^{-/-} and WT mice to ascertain whether the EP1-mediated pathway contributes to neuronal cell death. Results from the cell survival assays showed that the oxidative stressor, *t*-BuOOH (60 μ M), decreased WT neuronal viability by approximately 60% (cell viability: $39.6 \pm 17.2\%$) compared to vehicle-treated neurons. In contrast, viability of EP1^{-/-} neurons remained high (cell viability: $90.6 \pm 5.8\%$), with only a 10% reduction in viability compared to controls ($P < 0.05$ vs. WT) (Fig. 6). Moreover, cell survival was decreased significantly by PGE₂ (200 μ M) alone, an effect that was significantly attenuated by the EP1 antagonist SC-51089 (5 μ M) and by verapamil (10 μ M; $P < 0.05$) (Fig. 7). In addition, the increased toxicity caused by the combination of *t*-BuOOH with PGE₂ was significantly attenuated by both verapamil and SC-51089 ($P < 0.05$).

DISCUSSION

Our current study indicates that genetic deletion of the EP1 receptor results in significantly improved CBF in vivo and increased neuronal survival in vitro after toxic insults from MCAO and *t*-BuOOH, respectively. Our results also suggest a role for the EP1 receptor in blood pressure homeostasis. These data support our previously published report that infarct size was significantly smaller ($P < 0.01$) in EP1^{-/-} mice than in WT mice after 90 min of MCAO and 4 days of reperfusion (Ahmad et al., 2006). A substantial body of evidence supports a role for PGE₂ in the regulation of blood pressure and vascular tone. Recent reports have described the role of EP receptors in mediating the vascular action of PGE₂ (Zhang et al., 2000; Schweda and Kurtz, 2004). Li et al. (1997) showed that EP1 mediates PGE₂-induced vasoconstriction. Stimulation of EP1 by PGE₂ results in IP₃ generation and increases in cellular calcium via a G_{αq} protein-coupled mechanism (Watabe et al., 1993). It is likely that this increase in calcium generates contraction of smooth muscle cells that leads to vasoconstriction. In the EP1^{-/-} mice, EP3 signaling should be intact and able to mediate calcium flux and signaling, thereby affecting microvascular function. However, the EP2 and EP4 receptors might have counterbalancing effects by promoting vasodilatation. Our current results indicating that genetic deletion of EP1 enhances CBF is further supported by another report in which vasoconstriction induced by 17-PGE₂ and sulprostone was significantly attenuated by SC-19220 (an EP1-receptor antagonist) (Jadhav et al., 2004).

A previous study found that the absence of EP1 receptors in male mice was associated with a significant reduction in blood pressure (Audoly et al., 1999). However, we observed no significant difference in MABP between WT and EP1^{-/-} mice under halothane anesthesia (3.0% for induction, 1.0% for maintenance). CBF normally is well maintained in the brain through autoregulatory mechanisms that preserve a relatively constant blood flow despite changes in blood pressure (Liu et al., 2005) by varying vascular radius. As vessels constrict, pressure increases, and as vessels dilate, pressure decreases. Mechanisms involved in autoregulation include vasomotor (myogenic, adrenergic), flow-metabolism coupling, and metabolic (chemical: oxygen, carbon dioxide, pH) regulation. However, autoregulation is frequently impaired after a severe brain injury. The most common components to be affected are flow-metabolism coupling or uncoupling, and vasomotor autoregulation (Kirkness, 2005; Littlejohns and Bader, 2005).

Although laser-Doppler flowmetry measures the advent of occlusion and reperfusion and is an adequate marker of relative changes in CBF, it may not fully capture regional differences in CBF in the penumbra that can have important physiologic consequences in stroke. To ascertain whether the EP1^{-/-} mice sustained an intra-ischemic insult similar to that of WT mice, and to evaluate whether enhanced vasodilatation could be a mechanism that leads to a decrease in infarct size in EP1^{-/-} mice, absolute CBF was calculated by quantitative [¹⁴C]-IAP autoradiography at 60 min of MCAO. Potential differences in vascular responsiveness between EP1^{-/-} and WT mice were examined by quantifying absolute regional CBF in the different regions of brain.

Finally, to examine the role of EP1 receptor on neuronal survival specifically, we measured the cell viability and found that EP1^{-/-} cortical neurons were more tolerant to *t*-BuOOH-induced oxidative stress than were the WT neurons. Our results support those of an earlier report that showed that blocking the prostanoid EP1 receptor during oxygen-glucose deprivation reduced Ca²⁺ and neurotoxicity (Gendron et al., 2005). The improved neuronal survival might result from changes in intracellular Ca²⁺. Supporting this possibility, our ongoing results suggest that both verapamil and the EP1 antagonist SC-51089 can attenuate the combined toxic effect of PGE₂- and *t*-BuOOH-induced cell death. These data suggest that EP1 receptors are likely to contribute to neurotoxicity at least partially by eliciting an increase in intracellular calcium. Furthermore, in an in vivo study that we reported previously, pretreatment with the EP1 receptor agonist ONO-DI-004 exacerbated the excitotoxic lesion induced by NMDA, whereas the selective EP1 receptor antagonist ONO-8711 attenuated the lesion (Ahmad et al., 2006). Similarly, genetic deletion of the EP1 receptor reduced the infarct size in ischemia-reperfusion brain injury (Ahmad et al., 2006; Kawano et al., 2006). Our data suggest that EP1 can significantly exacerbate brain damage and reduce neuronal cell survival.

In summary, we found that CBF in vivo and neuronal cell death in vitro were both affected by deletion of the EP1 receptor. These results suggest that the EP1 receptor might affect stroke outcome by CBF regulation as well as by direct action on neuronal survival. Therefore, EP1 receptor antagonists might be able to provide protection against cerebral ischemic injury.

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REFERENCES

- Ahmad AS, Saleem S, Ahmad M, Doré S. Prostaglandin EP1 receptor contributes to excitotoxicity and focal ischemic brain damage. *Toxicol Sci* 2006;89:265–270. [PubMed: 16237196]
- Audoly LP, Tilley SL, Goulet J, Key M, Nguyen M, Stock JL, McNeish JD, Koller BH, Coffman TM. Identification of specific EP receptors responsible for the hemodynamic effects of PGE₂. *Am J Physiol* 1999;277:H924–930. [PubMed: 10484412]
- Davis RJ, Murdoch CE, Ali M, Purbrick S, Ravid R, Baxter GS, Tilford N, Sheldrick RL, Clark KL, Coleman RA. EP4 prostanoid receptor-mediated vasodilatation of human middle cerebral arteries. *Br J Pharmacol* 2004;141:580–585. [PubMed: 14744815]
- Doré S, Otsuka T, Mito T, Sugo N, Hand T, Wu L, Hurn PD, Traystman RJ, Andreasson K. Neuronal overexpression of cyclooxygenase-2 increases cerebral infarction. *Ann Neurol* 2003;54:155–162. [PubMed: 12891667]
- Echeverria V, Clerman A, Doré S. Stimulation of PGE₂ receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following β -amyloid exposure. *Eur J Neurosci* 2005;22:2199–2206. [PubMed: 16262658]

- Funk CD, Furci L, FitzGerald GA, Grygorczyk R, Rochette C, Bayne MA, Abramovitz M, Adam M, Metters KM. Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. *J Biol Chem* 1993;268:26767–26772. [PubMed: 8253813]
- Gendron TF, Brunette E, Tauskela JS, Morley P. The dual role of prostaglandin E(2) in excitotoxicity and preconditioning-induced neuroprotection. *Eur J Pharmacol* 2005;517:17–27. [PubMed: 15964567]
- Guay J, Bateman K, Gordon R, Mancini J, Riendeau D. Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. *J Biol Chem* 2004;279:24866–24872. [PubMed: 15044444]
- Jadhav V, Jabre A, Lin SZ, Lee TJ. EP1- and EP3-receptors mediate prostaglandin E2-induced constriction of porcine large cerebral arteries. *J Cereb Blood Flow Metab* 2004;24:1305–1316. [PubMed: 15625406]
- Jay TM, Lucignani G, Crane AM, Jehle J, Sokoloff L. Measurement of local cerebral blood flow with [¹⁴C]iodoantipyrine in the mouse. *J Cereb Blood Flow Metab* 1988;8:121–129. [PubMed: 3339102]
- Kawano T, Anrather J, Zhou P, Park L, Wang G, Frys KA, Kunz A, Cho S, Orio M, Iadecola C. Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. *Nat Med* 2006;12:225–229. [PubMed: 16432513]
- Kirkness CJ. Cerebral blood flow monitoring in clinical practice. *AACN Clin Issues* 2005;16:476–487. [PubMed: 16269893]
- Li DY, Abran D, Peri KG, Varma DR, Chemtob S. Inhibition of prostaglandin synthesis in newborn pigs increases cerebral microvessel prostaglandin F2 alpha and prostaglandin E2 receptors, their second messengers and vasoconstrictor response to adult levels. *J Pharmacol Exp Ther* 1996;278:370–377. [PubMed: 8764372]
- Li DY, Hardy P, Abran D, Martinez-Bermudez AK, Guerguerian AM, Bhattacharya M, Almazan G, Menezes R, Peri KG, Varma DR, Chemtob S. Key role for cyclooxygenase-2 in PGE2 and PGF2alpha receptor regulation and cerebral blood flow of the newborn. *Am J Physiol* 1997;273:R1283–1290. [PubMed: 9362291]
- Littlejohns L, Bader MK. Prevention of secondary brain injury: targeting technology. *AACN Clin Issues* 2005;16:501–514. [PubMed: 16269895]
- Liu J, Simpson DM, Allen R. High spontaneous fluctuation in arterial blood pressure improves the assessment of cerebral autoregulation. *Physiol Meas* 2005;26:725–741. [PubMed: 16088064]
- Nakayama Y, Omote K, Kawamata T, Namiki A. Role of prostaglandin receptor subtype EP1 in prostaglandin E2-induced nociceptive transmission in the rat spinal dorsal horn. *Brain Res* 2004;1010:62–68. [PubMed: 15126118]
- Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 2001;108:25–30. [PubMed: 11435452]
- Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 1999;79:1193–1226. [PubMed: 10508233]
- Norel X, de Montpreville V, Brink C. Vasoconstriction induced by activation of EP1 and EP3 receptors in human lung: effects of ONO-AE-248, ONO-DI-004, ONO-8711 or ONO-8713. *Prostaglandins Other Lipid Mediat* 2004;74:101–112. [PubMed: 15560119]
- Oka T. Prostaglandin E2 as a mediator of fever: the role of prostaglandin E (EP) receptors. *Front Biosci* 2004;9:3046–3057. [PubMed: 15353336]
- Rocca B. Targeting PGE2 receptor subtypes rather than cyclooxygenases: a bridge over troubled water? *Mol Interv* 2006;6:68–73. [PubMed: 16565468]
- Sampei K, Goto S, Alkayed NJ, Crain BJ, Korach KS, Traystman RJ, Demas GE, Nelson RJ, Hurn PD. Stroke in estrogen receptor-alpha-deficient mice. *Stroke* 2000;31:738–743. [PubMed: 10700513] discussion 744
- Sawada M, Alkayed NJ, Goto S, Crain BJ, Traystman RJ, Shaivitz A, Nelson RJ, Hurn PD. Estrogen receptor antagonist ICI182,780 exacerbates ischemic injury in female mouse. *J Cereb Blood Flow Metab* 2000;20:112–118. [PubMed: 10616799]
- Schweda F, Kurtz A. Cellular mechanism of renin release. *Acta Physiol Scand* 2004;181:383–390. [PubMed: 15283749]

- Stock JL, Shinjo K, Burkhardt J, Roach M, Taniguchi K, Ishikawa T, Kim HS, Flannery PJ, Coffman TM, McNeish JD, Audoly LP. The prostaglandin E2 EP1 receptor mediates pain perception and regulates blood pressure. *J Clin Invest* 2001;107:325–331. [PubMed: 11160156]
- Suganami T, Mori K, Tanaka I, Mukoyama M, Sugawara A, Makino H, Muro S, Yahata K, Ohuchida S, Maruyama T, Narumiya S, Nakao K. Role of prostaglandin E receptor EP1 subtype in the development of renal injury in genetically hypertensive rats. *Hypertension* 2003;42:1183–1190. [PubMed: 14670979]
- Watabe A, Sugimoto Y, Honda A, Irie A, Namba T, Negishi M, Ito S, Narumiya S, Ichikawa A. Cloning and expression of cDNA for a mouse EP1 subtype of prostaglandin E receptor. *J Biol Chem* 1993;268:20175–20178. [PubMed: 7690750]
- Wong D, Wang M, Cheng Y, Fitzgerald GA. Cardiovascular hazard and non-steroidal anti-inflammatory drugs. *Curr Opin Pharmacol* 2005;5:204–210. [PubMed: 15780832]
- Zhang Y, Guan Y, Schneider A, Brandon S, Breyer RM, Breyer MD. Characterization of murine vasopressor and vasodepressor prostaglandin E(2) receptors. *Hypertension* 2000;35:1129–1134. [PubMed: 10818076]
- Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* 2003a; 6:43–50. [PubMed: 12469126]
- Zonta M, Sebelin A, Gobbo S, Fellin T, Pozzan T, Carmignoto G. Glutamate-mediated cytosolic calcium oscillations regulate a pulsatile prostaglandin release from cultured rat astrocytes. *J Physiol* 2003b; 553:407–414. [PubMed: 14500777]

Fig. 1. Genetic deletion of EP1 does not significantly affect cerebral arterial vasculature in mice. Macroscopic analysis of cerebral arterial vasculature did not show differences in the circle of Willis or major cerebral arteries between EP1^{-/-} and WT mice ($n = 5$).

Fig. 2.

Mice with genetic deletion of EP1 receptor have enhanced cerebral blood flow (CBF) at reperfusion. Decreases in cortical perfusion during ischemia were estimated by laser-Doppler flowmetry using a flexible fiber optic probe affixed to the skull over the parietal cortex supplied by the middle cerebral artery. Percent change from baseline was recorded at baseline, at induction of ischemia, and at 15-min intervals during ischemia and for 1 hr of reperfusion in WT and EP1^{-/-} mice ($n = 5$). * $P < 0.05$.

Fig. 3.

No significant differences in body temperature were observed in WT and EP1^{-/-} mice. Body temperature was recorded at baseline, at induction of ischemia, and at 15-min intervals during ischemia and for 1 hr of reperfusion in WT and EP1^{-/-} mice ($n = 5$).

Fig. 4. Genetic deletion of EP1 receptor does not affect mean arterial blood pressure (MABP). MABP was recorded at baseline, at induction of ischemia, and at 15-min intervals during ischemia and for 1 hr of reperfusion in WT and EP1^{-/-} mice ($n = 5$).

Fig. 5. Quantification of regional cerebral blood flow (CBF) in WT (left) and EP1^{-/-} (right) mice. Regional CBF was determined by [¹⁴C]-IAP autoradiography within five regions of contralateral nonischemic cortex, ipsilateral ischemic cortex, and caudate putamen, subdivided into parietal, lateral, and medial areas, at 60 min of middle cerebral artery occlusion. *n* = 4 mice per group. The CBF color key to the right indicates that colors toward the red spectrum are interpreted as areas of higher flow, whereas colors toward the green/blue spectrum are areas of lower flow. P1 CTX, parietal 1 cortex; CP1 CTX, contralateral parietal 1 cortex; P2 CTX, parietal 2 cortex; CP2 CTX, contralateral parietal 2 cortex; LAT CTX, lateral cortex; CLAT CTX, contralateral lateral cortex; DM CP, dorsomedial caudate putamen; CDM CP, contralateral dorsomedial caudate putamen; VL CP, ventrolateral caudate putamen; CVL CP, contralateral ventrolateral caudate putamen. ***P* < 0.01.

Fig. 6. Genetic deletion of EP1 receptor reduces *t*-BuOOH-induced neuronal cell death. Neuronal cell cultures were exposed to *t*-BuOOH for 24 hr, after which viability was determined. Cell viability was significantly higher in the neurons derived from EP1^{-/-} mice than in those derived from WT mice. *n* = 3; **P* < 0.05.

Fig. 7.

EP1 antagonist SC-51089 (SC) and the calcium channel blocker verapamil protect neuronal cells exposed to *t*-BuOOH. Neurons were treated for 24 hr in either culture medium alone (control; CTL), or in the presence of 60 μ M *t*-BuOOH, 200 μ M PGE₂, 5 μ M SC, 10 μ M verapamil, or combinations, and cell viability was determined. #*P* < 0.05 vs. control; **P* < 0.05 vs. PGE₂; †*P* < 0.05 vs. *t*-BuOOH+PGE₂.

TABLE I

Effect of MCAO on Physiologic Parameters in EPI^{-/-} and Wild-Type Mice

Parameter	Wild-type mice			EPI ^{-/-} mice		
	Baseline	MCAO (1 hr)	Reperfusion (1 hr)	Baseline	MCAO (1 hr)	Reperfusion (1 hr)
pH	7.36 ± 0.02	7.34 ± 0.01	7.35 ± 0.01	7.37 ± 0.03	7.33 ± 0.01	7.35 ± 0.02
P _a CO ₂	38.5 ± 1.1	40.5 ± 1.2	39.0 ± 1.1	40.4 ± 1.0	40.9 ± 1.2	41.7 ± 1.2
P _a O ₂	105 ± 3	131 ± 3	107 ± 2	104 ± 2	114 ± 2 *	109 ± 2

Values are mean ± SEM of five animals.

* *P* < 0.05 compared with wild-type group.