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Reduced acute brain injury in PGE₂ EP3 receptor–deficient mice after cerebral ischemia

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Abstract

Ischemic stroke is one of the leading causes of mortality and morbidity in humans. During brain ischemia and the subsequent reperfusion that occurs with stroke, the generation of the so-called “proinflammatory” prostaglandin E₂ (PGE₂) increases significantly. Therefore, interest is growing regarding the differential functions of the individual PGE₂ receptors (EP1–4) and their relative contribution to brain damage following ischemic and inflammatory stimuli. Here, we address the contribution of the EP3 receptor in dictating early outcomes after transient cerebral ischemia. An oxygen-glucose deprivation (OGD)-induced *in vitro* model of brain ischemia was used in mouse hippocampal slice cultures. For transient ischemia, the right middle cerebral artery (MCA) of wildtype (WT) and EP3 knockout (EP3^{−/−}) C57BL/6 male mice was occluded for 90 min and perfused for 48 or 96 h, after which neurobehavioral scores and infarct volumes were determined. Mean arterial blood pressure, pH, blood gases (PaO₂ and PaCO₂), cerebral blood flow, and body temperature were also determined before and during ischemia and reperfusion. OGD-induced cell death was significantly lower in brain slice cultures of EP3^{−/−} mice than in those of WT mice. EP3^{−/−} mice that underwent transient ischemia had significantly smaller infarct volumes than did WT mice at 48 h, but this difference was not sustained at 96 h. Neurological score deficits correlated with infarct volume, but no significant differences in the physiological parameters monitored were detected between the two genotypes. The results further support a role for EP3 receptors in contributing to acute ischemic stroke, but EP3 is not likely the sole contributor to the long-term detrimental consequences of PGE₂.

Keywords

EP3 receptor; focal cerebral ischemia; oxygen glucose deprivation; prostanoids

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1. Introduction

Increasing evidence indicates that oxidative stress and delayed inflammatory processes play a crucial role in ischemic-reperfusion (I/R) injury. The generation of reactive oxygen species as a result of the early reperfusion period causes direct neurotoxic effects, including lipid peroxidation and oxidative protein and DNA damage (Muralikrishna Adibhatla and Hatcher, 2006). Brain damage may also occur as a result of increased free radical production during the post-reperfusion inflammatory response (Hoehn et al., 2005; Huang et al., 2006). One determinant of inflammation-associated cytotoxicity is the increased expression of cyclooxygenase (COX)-2 (Iadecola and Gorelick, 2005; Kawano et al., 2006). COX catalyzes the conversion of arachidonic acid to prostaglandin (PG) H₂, after which, specific PG synthases metabolize PGH₂ to at least five structurally related bioactive lipid molecules, including PGE₂, PGD₂, PGF_{2α}, PGI₂, and thromboxane A₂ (Doré, 2006; Wang et al., 2005). Previous studies showed that COX-2-deficient mice and mice given COX-2 inhibitor had smaller infarct volumes after ischemic insult than did wildtype (WT) or vehicle-treated mice, respectively (Iadecola et al., 2001; Sugimoto and Iadecola, 2003). Conversely, transgenic mice that overexpressed COX-2 had larger infarct volumes than did nontransgenic mice (Doré et al., 2003).

It has been documented that PGE₂ mediates many, if not most, of the COX-2-induced neurotoxicity in inflammation and neurological disorders (Takadera and Ohyashiki, 2006). PGE₂ exerts its cellular effects by binding primarily to high affinity receptors designated EP1 through EP4, which belong to the family of G-protein-coupled receptors. Stimulation of the EP1 receptor results in activation of phosphatidyl inositol hydrolysis and elevation of the intracellular Ca²⁺ concentration (Ahmad et al., 2006a; Kawano et al., 2006). EP2 and EP4 activation increase intracellular cyclic adenosine monophosphate (cAMP), whereas EP3 activation, in most instances, decreases it. EP3, like EP1, can mediate Ca²⁺ flux and signaling (Audoly et al., 1999a). Thus, the potential is great for variability in the response of target cells to PGE₂ based on which receptors it activates (Narumiya et al., 1999).

The various roles of the PGE₂ receptors in acute and chronic neurodegenerative diseases have been clarified by experiments that used receptor knockout mice and selective receptor agonists and antagonists. We and others have documented that mice lacking the EP1 receptor are partially resistant to neuronal damage caused by excitotoxicity and ischemic stroke (Ahmad et al., 2006a; Kawano et al., 2006). Moreover, in neuronal cells given NMDA, an EP1 receptor antagonist decreased the excitotoxicity, whereas treatment with an EP1 receptor agonist increased toxicity (Gendron et al., 2005). Knockout of the EP2 gene reduced the infarct volume in male mice after ischemic stroke (Liu et al., 2005; McCullough et al., 2004). It has been shown that an EP4 receptor agonist reduces excitotoxic and I/R-induced brain injury (Ahmad et al., 2005; Ahmad et al., 2006b) and that agonists of EP2 and EP4 decrease neuronal cell death induced by β-amyloid toxicity (Echeverria et al., 2005). Interestingly, we have previously documented that stimulation of the EP3 receptor with the selective agonist ONO-AE-248 exacerbates acute excitotoxic or ischemic-induced brain injury (Ahmad et al., 2007), whereas others have suggested that EP3 could be protective by using either a moderately selective agonist or a tissue-specific EP3 overexpression system (Bilak et al., 2004; Martin et al., 2005). Activation of the EP3 receptor also causes vasoconstriction and has been described to have a significant role in hyperthermia (Jadhav et al., 2004; Nakamura et al., 2005). The EP3 receptor mRNA has been documented in the central nervous system (CNS); it is found highly expressed in layer V of the cortex, in the pyramidal layer of the hippocampus, in the thalamus, and in the median preoptic nucleus (Allen Brain Atlas [Internet], 2007; Ek et al., 2000). Here, using mice with a genetic deletion for EP3, we observed that hippocampal slice cultures prepared from these EP3 knockout (EP3^{-/-}) mice were protected against neuronal damage induced by the oxygen-glucose deprivation (OGD) model. We then investigated the importance

of the EP3 receptor in the CNS in regard to the outcomes of transient focal cerebral ischemia and found that it contributes to acute but not necessarily long-term outcomes.

2. Materials and methods

2.1. Experimental animals

This study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. Experimental protocols were approved by the Johns Hopkins Institutional Animal Care and Use Committee. Adult male EP3^{-/-}C57BL/6 mice and WT littermates were originally provided by Dr. Shuh Narumiya and are maintained and genotyped in our animal colonies.

2.2. Preparation of mouse hippocampal organotypic slice cultures

Hippocampal organotypic cultures were performed as described in previous study (Ahmad et al., 2008). Hippocampi from 7–8-day-old C57BL/6 mouse pups were dissected out aseptically, and 350-μm sections were obtained with a vibratome (Series 1000, Harvard Apparatus, Holliston, MA, USA) and stored in Hibernate A solution (BrainBits, Springfield, IL, USA) bubbled with 95% O₂ and 5% CO₂. Slices were transferred onto 30-mm Millicell membrane inserts with 0.4 μm pore size (Millipore, Bedford, MA) in six-well plates containing Neurobasal A medium with B27 supplement and 2 mM GlutaMAX-I (Invitrogen, Carlsbad, CA). Cultures were maintained for 13 days in a humidified, 5% CO₂ atmosphere at 37°C; medium was changed every 2 to 3 days. On day 13, the medium was replaced with fresh medium containing propidium iodide (PI; 4.5 μg/ml; Sigma).

2.3. Oxygen-glucose deprivation and measurement of neuronal death in hippocampal slices

OGD prepared according to the protocol described by Ahmad et al., 2008 and Zhou et al., 2008. Anoxia was induced by subjecting the slices to OGD for 1 h. First, slice cultures were rinsed with normal medium and equilibrated for 30 min at 37°C and then rinsed twice with warm deoxygenated glucose-free Hibernate A solution. The cultures were then transferred to an air-tight 37°C-chamber, and anoxic gas (5% CO₂, 95% N₂) was flushed into the chamber for 1 h. After OGD, the slices were transferred to normal culture medium and incubated for 24 h. Cultures were imaged for PI fluorescence 24 h after OGD and then, to obtain maximal PI fluorescence, they were stimulated for 24 h with a lethal dose of NMDA (100 μM) and imaged. For measurement of cell death, mean PI fluorescence in the CA1 subregion of each hippocampal slice was quantified. Sequential fluorescence was measured (1) before OGD stimulation to measure basal levels of neuronal death, $t = 0$ (F_{basal}); (2) 24 h after stimulation with OGD, $t = 24$ h (F_{OGD}); and (3) after a final exposure to 100 μM NMDA overnight, $t = \text{max}$ (F_{max}). Slices were imaged with a Nikon fluorescence microscope equipped with a light-intensifying SPOT digital camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA) and SPOT Advanced software. The same camera settings were used throughout experiments. Digital images of PI staining at the three time points (F_{basal} , F_{OGD} and F_{max}) were outlined to define the same CA1 region and mean fluorescence intensity obtained at the corresponding time points. The percent cell death was calculated by the formula: $(F_{\text{OGD}} - F_{\text{basal}}) / (F_{\text{max}} - F_{\text{basal}}) \times 100\%$.

2.4. Measurement of physiological parameters

In a separate cohort of WT and EP3^{-/-} mice ($n = 5/\text{group}$), the femoral artery was cannulated to measure arterial blood gases (pH, PaO₂, and PaCO₂) and mean arterial blood pressure (MABP) at baseline and every 15 min during the 90 min of ischemia and 60 min of reperfusion.

2.5. Telemetric temperature monitoring

Under halothane anesthesia (3.0% for induction, 1.0% for maintenance) and ventilation with oxygen-enriched air via a nose cone, animals were implanted with a radio telemetric probe (IPTT-200, BMDS, Seaford, DE, USA) to enable measurement of core body temperature. Animals were allowed to recover for ≥ 7 days before experiments were performed. Body temperature was measured via receivers of a telemetry system (DAS-5002, BMDS) that minimizes stress and allows temperature monitoring in freely moving animals. Body temperature was determined in the transient ischemic groups at baseline and at 15-min intervals during the course of the experiments and up to 96 h of reperfusion.

2.6. Transient focal cerebral ischemia model

Transient focal cerebral ischemia was induced by middle cerebral artery (MCA) occlusion (MCAO) with an intraluminal filament technique. Adult male mice (20–25 g at 8–10 weeks old) were placed under halothane anesthesia (3.0% for induction, 1.0% for maintenance) and ventilated with oxygen-enriched air via a nose cone. Body temperatures were maintained at $37.0 \pm 0.5^\circ\text{C}$ by a heating pad. Relative cerebral blood flow (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). Under aseptic conditions, the neck and carotid bifurcation were dissected. The common carotid artery was temporarily ligated, and the external carotid artery was used as a stump. A 7-0 Ethilon nylon monofilament (Ethicon, Inc., Somerville, NJ) coated with flexible silicone and universal hardener (Cutter Sil, Heraeus Kulzer GmbH, Hanau, Germany) was used to occlude the MCA. After an incision was made in the external carotid artery stump, the filament was advanced through the internal carotid artery to the origin of the MCA; cessation of blood flow was documented by a decrease in laser-Doppler signal. The neck then was sutured and closed and anesthesia was discontinued. The mice were transferred to a temperature-controlled chamber to maintain body temperature at $37.0 \pm 0.5^\circ\text{C}$ while the filament was left in position for 90 min. To initiate reperfusion, the mice were briefly anesthetized with halothane, and the filament was withdrawn to reopen the MCA. The neck was sutured, and the mice were returned to the temperature-controlled chamber for approximately 2 h before being returned to room temperature.

2.7. Neurological evaluation

At 48 h or 96 h after transient MCAO, mice were tested for neurological deficits before being sacrificed for infarct measurement. Neurological function was graded on the following scoring system (Li et al., 2004; Saleem et al., 2007): 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.

2.8. Quantification of infarct volume

Mice were anesthetized deeply, and the brains were harvested and sliced coronally into five 2-mm-thick sections, which were incubated with 1% 2,3,5-triphenyltetrazolium chloride (TTC) in saline for 30 min at 37°C . The area of brain infarct, identified by the lack of TTC staining, was measured on the rostral and caudal surfaces of each slice and numerically integrated across the thickness of the slice to obtain an estimate of infarct volume (Sigma Scan Pro, Systat, Port Richmond, CA). Volumes from all five slices were summed to calculate total infarct volume over the entire hemisphere, expressed as a percentage of the volume of the contralateral hemisphere. Infarct volume was corrected for swelling by comparing the volumes in the ipsilateral and contralateral hemispheres. The corrected volume of the infarcted hemisphere was calculated as volume of contralateral hemisphere – (volume of ipsilateral hemisphere – infarct volume) (Doré et al., 2003).

2.9. Statistical analysis

Data are expressed as means $\pm$ S.E.M. Multiple comparisons were analyzed by the ANOVA and Tukey's test, with significance set at $P < 0.05$.

3. Results

3.1. EP3 deletion reduces the neuronal damage in the CA1 region of hippocampal slices

To study the effect of EP3 receptor in an *in vitro* model of ischemia, organotypic hippocampal slices were exposed to 1 h of OGD. Cell death was measured via PI labeling (Fig. 1A). No significant difference in $F_{\max}$ between WT and EP3^{-/-} group was observed. The analysis indicated a significant decrease in cell death after OGD in the EP3^{-/-} group as compared with that in the WT group ($44.7 \pm 2.0\%$ vs $54.3 \pm 1.9\%$, $P < 0.01$, Fig. 1B). These results indicate that the EP3 receptor plays a role in contributing to acute ischemia-induced hippocampal cell death, at least *in vitro*.

3.2. Cerebral blood flow and physiological parameters were not altered before, during, or after ischemic stroke in the EP3^{-/-} mice

We used laser-Doppler flowmetry measurements to assess whether the ischemic insult was similar in all animal groups. We observed that the relative CBF of WT and EP3^{-/-} mice decreased to less than 20% of baseline after MCAO and returned to near baseline after reperfusion was initiated. No differences in CBF were observed between the two genotypes of mice (Fig. 2A). Physiological parameters of pH, PaO₂, PaCO₂, and MABP were also unchanged during the course of ischemia and reperfusion and were not significantly different between WT and EP3^{-/-} mice (Table 1, Fig. 2B).

3.3. Body temperature was not altered before, during, or after ischemic stroke in the EP3^{-/-} mice

Considering the reported potential role of EP3 in controlling core temperature, in a separate cohort of mice, we recorded body temperature during ischemia and during reperfusion and found no detectable differences between the WT and EP3^{-/-} mice (Fig. 3).

3.4. EP3 deletion lessens neurological deficit score and brain infarct size after ischemic-reperfusion injury

Before sacrificing the animals, neurological deficit scores were determined. The neurological scores of the EP3^{-/-} mice were significantly lower than those of the WT mice 48 h after transient ischemic insult (1.67 ± 0.14 vs 2.50 ± 0.27 , $P < 0.05$), indicating better functional ability (Fig. 4A). However, 96 h after transient ischemia, all mice had a neurological score of ≥ 2 , with no significant difference between EP3^{-/-} and WT mice (2.25 ± 0.18 vs 2.33 ± 0.17). In addition, the cerebral infarct volume of the EP3^{-/-} mice was significantly smaller than that of the WT mice at 48 h (30.8 ± 7.4 vs 48.1 ± 5.8 , $P < 0.05$), but the difference was no longer significant at 96 h (35.0 ± 6.9 vs 45.0 ± 4.6) (Figs. 4B and 4C).

4. Discussion

In this study, using EP3^{-/-} mice, we found that EP3 receptors might have a negative effect on neurological function and infarct size in the short-term but that in the long-term, they have little impact. This result supports our earlier reports suggesting that in general, PGE₂ receptors that decrease cAMP or increase intracellular Ca²⁺ exacerbate injury (Ahmad et al., 2006a; Ahmad et al., 2006b). Whereas EP2 and EP4 have consistently been coupled to Gs, which is associated with an increase in cAMP levels, EP1 is thought to be coupled mainly to Gq, which affects inositol phosphate turnover and Ca²⁺. The G-proteins through which EP3 signals can

vary; thus, the role of EP3 in transient ischemic stroke is unclear. Attempting to shed light on the effect of the EP3 receptor, in the current study, we first observed that neuronal death after OGD protocol in EP3^{-/-} mouse hippocampal slice cultures was significantly attenuated than in cultures from WT mice. We then found that deletion of the EP3 gene provided some protection at 48 h, but by 96 h had no effect on I/R-induced brain injury.

Previously we have reported that an EP3-selective agonist could exacerbate the toxic effects of acute NMDA-induced excitotoxicity and I/R injury (Ahmad et al., 2007). In addition, it was reported that the EP3 receptor enhanced excitotoxicity in rat striatum (Slawik et al., 2004). In contrast, another study suggested that cardiospecific overexpression of EP3 receptors decreases myocardial injury (Martin et al., 2005). Here, our data also indicate that EP3^{-/-} mice have reduced cell death after OGD toxicity as compared with that of WT mice.

Various groups have investigated the physiological consequences of the increase in PGE₂ that follows ischemia. For example, it was suggested that selective stimulation of EP3 would trigger vasoconstriction in porcine middle cerebral arteries (Hatae et al., 2002; Jadhav et al., 2004), and the PGE₂-mediated vasoconstrictive effect of EP3 receptor in porcine large arteries and kidney has been documented (Audoly et al., 2001; Jadhav et al., 2004). Audoly et al. (Audoly et al., 1999b) also investigated the impact of EP3 receptor deletion on the systemic circulation and reported that EP3 contributed significantly to the PGE₂-dependent regulation of arterial blood pressure. They showed that a vasodepressor response in male EP3^{-/-} mice was consistent with a constrictor action of EP3 receptors in the peripheral vasculature. The vasodepression might have been mediated through activation of Gi and a subsequent reduction in cAMP and/or stimulation of intracellular Ca²⁺ (Breyer et al., 2001). Under our experimental conditions, no significant difference was observed in CBF or MABP between the WT and EP3^{-/-} mice before, during, or after reperfusion.

Optimal antibodies against these EP receptors are still lacking, but through studies of mRNA expression it has been suggested that EP1, EP2, and EP4 receptors are found only in isolated neuronal cell groups while the EP3 receptors are constitutively expressed throughout the brain (Candelario-Jalil et al., 2005; Nakamura et al., 2000; Zhang and Rivest, 1999). EP3 has been suggested to be the most abundantly expressed PGE₂ EP receptor subtype in the brain (Nakamura et al., 2000; Oka et al., 2000) and has multiple splice variants. In humans, at least eight EP3 isoforms have been identified (Kotani et al., 1997). The signaling pathway initiated is quite complex and depends on which individual EP3 isoform is activated. For example, isoforms EP3-I, EP3-II, and EP3-III have been reported to decrease the cAMP level and increase intracellular Ca²⁺ levels (An et al., 1994), whereas EP3-VI can mediate either an increase or decrease in cAMP (An et al., 1994; Kotani et al., 2000). However, only few splice variants have been identified in mice (Irie et al., 1994; Sugimoto et al., 1993). It should also be noted that while the PGF_{2α} receptor (FP), the thromboxane A₂ (TP), and EP1 receptors share the same branch on the phylogenetic tree, the EP3 occupies a neighboring branch (Toh et al., 1995). With the possibility of great variation in signaling pathways in different cells and different brain regions, additional investigation is needed to address the potential contribution that each of the EP3 isoforms play in ischemic reperfusion injury.

We undertook this study to further elucidate the role of the EP3 receptor in hypoxic/ischemic damage. Although we showed that EP3 appears to play a role in early reperfusion (48 h), this effect was lost at a later time point (96 h). In a similar study, Li and colleagues (Li et al., 2008a) showed that there were no differences in infarct size or CBF in the EP3^{-/-} mice at the 24-h time point. Although we did not measure outcomes at 24 h, we did find small but significant differences in infarct volume and behavior at the 48-h time point. The differences between our study and that of Li et al. may reside in various factors. For example, we monitored CBF in all of our animals to ensure the most appropriate placement of the occlusion filament.

In addition, we consistently used a siliconized flexible filament that minimizes blood vessel damage and significantly reduces intracerebral hemorrhage and mortality (Shah et al., 2006). We also used halothane anesthesia to minimize the pre- and post-conditioning effects associated with other anesthetics, such as isoflurane (Lee et al., 2008; Li et al., 2008b). Despite the fact that the outcome differences we saw at 48 h had disappeared by 96 h might be due of increased infarct size over the period of time, the initial differences do support the results of our previous work in which a selective EP3 agonist exacerbated excitotoxicity and stroke outcomes in WT mice subjected to a similar stroke protocol (Ahmad et al., 2007). EP3 receptors were also suggested to be responsible for *in vivo* excitotoxicity injury in rodents following intrastriatal injection of quinolinic acid (Slawik et al., 2004). Our results also suggest that differences at early stage (48h) might be due to acute excitotoxicity injury.

The EP3 receptor has been described to play a role in regulation of body temperature. For example, it was shown that lipopolysaccharide can induce hypothermia in EP3^{-/-} mice but not in controls (Oka et al., 2003). Several reports also suggest that hyperthermia and hypothermia both affect stroke outcomes; hyperthermia was shown to exacerbate stroke injury, whereas tightly regulated hypothermia had a protective role (Barber et al., 2004). Therefore, we used radio telemetry to determine whether our stroke protocol would differentially affect the body temperatures of freely moving WT and EP3^{-/-} mice. However, the recorded temperature did not change significantly in either mouse genotype during 96 h of post-ischemic reperfusion.

Together, the novel *in vitro* results using the OGD protocol, combined with our previous and current results of this study indicate that immediately following a transient ischemic insult, the neurotoxicity of PGE₂ could be partially mediated by EP3 receptor activity whereas in the long-term, other prostanoid receptors (i.e. EP1) are likely to be responsible for the pro-toxicity effects of PGE₂.

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Fig. 1. Effect of EP3 receptor deletion on oxygen-glucose deprivation-induced cell death in the CA1 region of cultured hippocampal slices. (A) Fluorescence images showing PI staining in hippocampal slices subjected to oxygen-glucose deprivation (OGD) for 1 h. Images (magnification: 4×) were taken before OGD (Base), 24 h after OGD (OGD), and 24 h after NMDA incubation (MAX). (B) Histogram representing CA1 neuronal damage measured by PI fluorescence intensity. After OGD, the neuronal cell death in the CA1 region measured as PI intensity was significantly lower in cultured hippocampal slices from EP3^{-/-} mice (n = 22 slices) than in those from WT mice (n = 24 slices; **P < 0.01).

Fig. 2.

Genetic deletion of EP3 receptor does not affect cerebral blood flow or mean arterial blood pressure (MABP) before, during, or after ischemia. (A) Decreases in cortical perfusion were estimated by laser-Doppler flowmetry with a flexible fiber optic probe affixed to the skull over the parietal cortex supplied by the middle cerebral artery. Percent changes from baseline were recorded at baseline, at induction of ischemia, and at 15-min intervals during ischemia and 1 h of reperfusion in WT and EP3^{-/-} mice. At the same time points, MABP was monitored by cannulation of the femoral artery (B). n = 5/group.

Fig. 3.

Genetic deletion of EP3 receptor does not affect core body temperature before, during, or after ischemia. Body temperature was recorded by radio telemetry at baseline, at induction of ischemia, and at 15-min intervals during ischemia and for up to 4 days of reperfusion in WT and EP3^{-/-} mice. n = 5/group.

Fig. 4.

Effect of EP3 receptor deletion on neurological deficit and infarct volume after transient middle cerebral artery occlusion (MCAO). (A) At 48 and 96 h of post-MCAO reperfusion, the neurological function of WT mice (n = 9 for 48 h; n = 10 for 96 h) and EP3^{-/-} mice (n = 10 for 48 h; n = 12 for 96 h) was assessed. (B) Representative photographs of coronal sections from the brains of WT and EP3^{-/-} mice at 48 and 96 h of reperfusion. (C) The percent corrected infarct volume of WT and EP3^{-/-} mice was measured by TTC staining after 90 min of MCAO and 48 or 96 h of reperfusion. *P < 0.05.

Table 1
Effect of MCAO on physiological parameters in WT and EP3^{-/-} mice (n = 5/group)

Parameter	Wildtype mice			EP3 ^{-/-} mice		
	Baseline	1 h MCAO	1 h Reperfusion	Baseline	1 h MCAO	1 h Reperfusion
pH	7.37 ± 0.02	7.34 ± 0.02	7.35 ± 0.01	7.34 ± 0.05	7.33 ± 0.02	7.33 ± 0.01
PaCO ₂	38.2 ± 1.4	40.0 ± 1.4	38.7 ± 1.4	40.9 ± 0.7	40.2 ± 2.2	39.9 ± 1.5
PaO ₂	107 ± 2	131 ± 3	109 ± 4	120 ± 4	119 ± 5	119 ± 6