Published in final edited form as:

Neurotox Res. 2009 January ; 15(1): 62–70. doi:10.1007/s12640-009-9007-3.

PGF_{2α} FP Receptor Contributes to Brain Damage Following Transient Focal Brain Ischemia

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Abstract

Although some of the COX-2 metabolites and prostaglandins have been implicated in stroke and excitotoxicity, the role of prostaglandin F_{2α} (PGF_{2α}) and its FP receptor have not been elucidated in the pathogenesis of ischemic-reperfusion (I/R) brain injury. Here we investigated the FP receptor's contribution in a unilateral middle cerebral artery (MCA) occlusion model of focal cerebral ischemia in mice. The MCA in wild type (WT) and FP knockout (FP^{-/-}) C57BL/6 male mice was transiently occluded with a mono-filament for 90 min. After 96 h of reperfusion, the FP^{-/-} mice had 25.3% less neurological deficit ($P < 0.05$) and 34.4% smaller infarct volumes ($P < 0.05$) than those of the WT mice. In a separate cohort, physiological parameters were monitored before, during, and after ischemia, and the results revealed no differences between the groups. Because excitotoxicity is an acute mediator of stroke outcome, the effect of acute NMDA-induced neurotoxicity was also tested. Forty-eight hours after unilateral intrastriatal NMDA injection, excitotoxic brain damage was 20.8% less extensive in the FP^{-/-} mice ($P < 0.05$) than in the WT counterparts, further supporting the toxic contribution of the FP receptor in I/R injury. Additionally, we investigated the effect of post-treatment with the FP agonist latanoprost in mice subjected to MCA occlusion; such treatment resulted in an increase in neurological deficit and infarct size in WT mice ($P < 0.05$), though no effects were observed in the latanoprost-treated FP^{-/-} mice. Together, the results suggest that the PGF_{2α} FP receptor significantly enhances cerebral ischemic and excitotoxic brain injury and that these results are of importance when planning for

potential development of therapeutic drugs to treat stroke and its acute and/or long term consequences.

Keywords

13,14-Dihydro-17-phenyl-18,19,20-trinor-PGF_{2α}-isopropyl ester; Excitotoxicity; Latanoprost; Middle cerebral artery occlusion; Mouse; Neurodegeneration; Prostaglandin

Introduction

In different parts of the world, stroke is the leading cause of death and physical disability (Shinohara 2006). Preclinical models of transient cerebral ischemia-reperfusion (I/R) have shown that brain is highly prone to oxidative damage, inflammation, and loss of neuronal homeostasis (Manabe et al. 2004). Studies have consistently demonstrated that during stroke, increased glutamate release, intracellular Ca²⁺ accumulation, and formation of reactive oxygen species lead to oxidative stress and cell death (Endres and Dirnagl 2002; Xu et al. 2005). Furthermore, the excess glutamate and hyper-activation of its receptors result in activation of phospholipid enzymes, phospholipid hydrolysis, and arachidonic acid release (Doré et al. 2003; Kawano et al. 2006; Muralikrishna Adibhatla and Hatcher 2006).

Arachidonic acid gives rise to prostaglandins (PGs) by the consecutive actions of cyclooxygenase (namely, COX-1 and COX-2) and PG synthesis enzymes. The effects of PGs are complex, and the brain's response is diverse because of the different types of receptors that mediate PG activity. The prostanoids, PGD₂, PGE₂, PGF_{2α}, PGI₂, and TXA₂, mediate their effects mainly through their respective specific G-protein-coupled receptors termed DP, EP (EP1-4), FP, IP, and TP. Receptor binding leads to either activation or inhibition of adenylyl cyclase, stimulation of phospholipase C-induced phosphoinositide turnover, or mobilization of intracellular Ca²⁺ (Coleman et al. 1994; Narumiya et al. 1999; Sharif et al. 2003).

PGF_{2α}, which is synthesized from PGH₂ via PGF synthase (Suzuki-Yamamoto et al. 1999) plays a significant role in initiation of parturition (Sugimoto et al. 1997), renal function (Breyer and Breyer 2001), control of cerebral blood flow (CBF) autoregulation in newborn piglets (Chemtob et al. 1990b), contraction of arteries (Nakahata et al. 2006), and myocardial dysfunction (Takayama et al. 2005; Jovanovic et al. 2006). FP receptors have been previously demonstrated in mouse brains (Muller et al. 2000) and in brain synaptosomes of newborn pigs (Li et al. 1995). Although the critical biological functions listed above for PGF_{2α} are reported to be mediated through activation of the FP receptor (Hata and Breyer 2004; Jovanovic et al. 2006), the contribution of PGF_{2α} FP receptor activation in brain injury has not been investigated.

Based on its presence in the central nervous system and the physiological roles it plays in vascular function and elevation of intracellular Ca²⁺, we hypothesize that activation of the FP receptor subsequent to injury contributes to excitotoxic and cerebral ischemic damage. By first using FP knockout (FP^{-/-}) mice and following up with the FP agonist latanoprost in WT mice, the current study was designed to test the potential neurotoxic role of the FP

receptor in ischemic and excitotoxic brain damage. Here, we analyzed the neurological deficit and physiological and histological parameters in mice subjected to transient middle cerebral artery (MCA) occlusion (MCAO) and reperfusion to determine the effect of FP receptor activation. Furthermore, to extend the results from the ischemic-reperfusion injury model, which is characterized by excitotoxic damage and inflammation, we investigated the contribution of the FP receptor in an NMDA-induced acute excitotoxicity model. Together, the results provide novel insight into the unique properties of the FP receptor within the central nervous system.

Materials and Methods

Animals

This study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. Protocols were approved by the Johns Hopkins Institutional Animal Care and Use Committee. FP^{-/-} C57BL/6 breeding mice were first obtained from Dr. Narumiya (Sugimoto et al. 1997) and then maintained and genotyped at our animal facility at Johns Hopkins University. Adult male FP^{-/-} and WT mice (20–25 g; 8–10 weeks old) were used in this study. All measures were taken to minimize pain and discomfort to the mice.

Transient Ischemia Protocol

Wild type ($n = 11$) and FP^{-/-} ($n = 12$) mice were subjected to MCAO for 90 min followed by 96 h of reperfusion (Ahmad et al. 2006a). Mice 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. The mice were subjected to the intraluminal filament technique to produce the MCAO model of transient focal cerebral ischemia as we have described previously (Ahmad et al. 2006a). 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 bregma). The induction of MCAO was achieved when the relative CBF decreased more than 80% from baseline; mice for which the CBF did not decrease below that level were excluded from additional experiments. During occlusion, mice were kept in a humidity-controlled, 32°C chamber to help maintain a body core temperature of 37°C . After 90 min of occlusion, mice were briefly re-anesthetized, the midline was reopened, and the filament was removed to establish reperfusion. After the incision was sutured, mice were again placed in the humidity- and temperature-controlled chamber for another 6 h and finally returned to their respective cages for survival up to 96 h.

Measurement of Physiological Parameters

In a separate cohort of WT ($n = 5$) and FP^{-/-} ($n = 5$) mice, the femoral artery was cannulated for measurement of physiological parameters (pH, PaCO₂, and PaO₂) and mean arterial blood pressure (MABP). Physiological parameters were monitored at baseline, 1 h of ischemia, and 1 h after reperfusion, whereas CBF, MABP, and rectal temperature were monitored every 15 min before and during ischemia and for 1 h of reperfusion.

Assessment of Neurological Deficit Score

Neurological function was measured in each mouse at 96 h after reperfusion according to the following graded scoring system, as described previously (Li et al. 2004): 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.

Post-Treatment of Mice with the FP Agonist Latanoprost

We used the selective FP receptor agonist latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-PGF_{2α}-isopropyl ester; Cayman Chemicals, Ann Arbor, MI, USA) (Stjernschantz et al. 2000; Sharif et al. 2003), which is also used clinically (Abdel-Halim et al. 1977; Perry et al. 2003), to determine the role of FP receptor activation in ischemic brain damage. Latanoprost was supplied as a 10 mg/ml solution in methyl acetate. The methyl acetate was evaporated under nitrogen, and the residual latanoprost was immediately dissolved in 100 μl of DMSO and diluted with 0.9% saline to obtain a stock solution of 10 mg/ml. Subsequent doses were freshly made such that each mouse received a final concentration of 0.5% DMSO. WT mice were post-treated orally with 10 μg/kg ($n = 10$) or 100 μg/kg ($n = 8$) latanoprost 30 min after reperfusion. Vehicle-treated WT controls ($n = 6$) were given 0.5% DMSO (diluted with 0.9% saline). At 96 h after MCAO, neurological deficit scores and brain injury were measured. To further determine the specificity of latanoprost toward stroke outcome, we repeated the experiment in FP^{-/-} mice using vehicle ($n = 7$) and 100 μg/kg latanoprost ($n = 7$).

Assessment of Brain Infarction

After 96 h of reperfusion, mice were deeply anesthetized, and their brains were collected and sliced coronally into 2-mm-thick sections. Infarct volume was assessed by 2,3,5-triphenyltetrazolium chloride (TTC) staining by an observer blinded to the treatments, as described previously (Ahmad et al. 2006a). Infarct volume was corrected for swelling by comparing the volumes in the ipsilateral and contra-lateral hemispheres. The corrected volume of the infarcted hemisphere was calculated as: volume of contralateral hemisphere – (volume of ipsilateral hemisphere – volume of infarct).

Unilateral NMDA-Induced Acute Excitotoxicity

To investigate the effect of FP on NMDA toxicity, WT and FP^{-/-} mice ($n = 7$ /group) were injected in the right striatum with 15 nmol NMDA (in 0.3 μl) or vehicle as described before (Ahmad et al. 2006a). After injection, the hole was blocked and the skin sutured, and the animals were transferred to a humidity- and temperature-controlled chamber. After full recovery from anesthesia, the mice were transferred to their home cages and were allowed to survive for 48 h. Throughout the stereotactic procedure, the rectal temperature of the mice was monitored and maintained at $37.0 \pm 0.5^\circ\text{C}$.

Quantification of the Excitotoxic Lesion Volume

Forty-eight hours after NMDA injection, weight and rectal temperature were recorded, and the mice were deeply anesthetized with pentobarbital sodium (65 mg/kg). The mice then

were perfused transcardially with cold PBS followed by 4% paraformaldehyde (pH 7.2) in PBS. Brains were removed quickly and post-fixed overnight before being equilibrated in sucrose (30%) and frozen in 2-methyl butane (pre-cooled over dry ice). Sequential brain sections (25 μ m) were cut on a cryostat, and every fourth section was stained with Cresyl violet to estimate lesion volume, as described previously (Ahmad et al. 2006b). Briefly, every stained section was imaged with computerized SigmaScan Pro 5.0 (Systat, Inc., Point Richmond, CA, USA) software. The entire lesion (recognized as a lightly stained area with extensive cell loss in the ipsilateral striatum) was encircled, enabling the software to calculate the lesion area in pixels. The area in pixels of every stained section was summed and divided by the total number of sections to obtain the mean lesion area in pixels. A piece of graph paper was also photographed; a square area was measured in pixels to convert the lesion area from pixels to mm². Then, the lesion area in mm² was multiplied by the section thickness to determine the lesion volume in mm³. In different groups, the number of sections with lesions ranged from 50 to 80.

Statistical Analysis

The brain sections were imaged and analyzed with SigmaScan Pro 5.0 software (Systat, Inc., Point Richmond, CA, USA); SigmaStat 3.11 was used for statistical analysis. We used two-way ANOVA followed by Bonferroni multiple comparison test to determine the difference in the physiological parameters between two groups at a given time point. One-way ANOVA followed by Bonferroni multiple comparison test was used to determine the difference in brain damage after MCAO in WT and FP^{-/-}, latanoprost-treated groups, and after NMDA injection in WT and FP^{-/-} mice. All values are expressed as mean $\pm$ standard error of the mean (SEM). The distribution of neurological deficit scores were compared across treatment groups using Fisher exact tests. Values of $P < 0.05$ were considered to be significant.

Results

Genetic Deletion of FP Receptors Does not Affect the Vital Physiological Parameters in Mice

We detected no significant differences in blood gases mice during (PaO₂, PaCO₂) or pH between WT and FP^{-/-} MCAO or after reperfusion (Table 1). The relative CBF decreased to >80% from baseline in both groups after MCAO and returned to near baseline after reperfusion was achieved. No substantial differences in CBF, body temperature, or MABP were observed between the two groups before, during, or after MCAO (Fig. 1).

Reduced Neurological Deficits and Brain Infarction in FP^{-/-} Mice After Transient Ischemia

At 96 h after the occlusion, the proportion of FP^{-/-} mice with neurological deficit was significantly lower than those of the WT mice ($P < 0.05$; Table 2). In addition, analysis of the brain slices stained with TTC showed that FP^{-/-} mice had significant attenuation of brain infarction volume as compared with similarly treated WT mice (62.8 ± 11.6 vs. 95.8 ± 10.7 mm³; $P < 0.05$; Fig. 2a, b).

NMDA-Induced Neurotoxicity is Reduced in FP^{-/-} Mice

To further investigate the contribution of FP receptor in the pathology of stroke, we investigated whether FP^{-/-} mice would be protected against acute excitotoxicity induced by NMDA. Cresyl violet-stained brain sections revealed that the NMDA-induced lesion volume was significantly smaller in the FP^{-/-} mice than in the WT mice (5.1 ± 0.5 vs. 6.5 ± 0.3 mm³; $P < 0.05$; Fig. 3).

Activation of FP Receptor by Agonist Latanoprost Augments Stroke Outcome in WT but not in FP^{-/-} Mice

To take into consideration, the potential compensatory mechanisms in knockout animals and further confirm the role of the FP receptor in stroke, we investigated the effect of post-treatment with the FP receptor agonist latanoprost in WT and FP^{-/-} mice subjected to MCAO. The WT cohort treated with 10 µg/kg latanoprost showed a trend toward increased neurological deficit, although the effect was not statistically significant when compared with the vehicle-treated mice. However, the neurological deficit increased significantly in the group that was post-treated with 100 µg/kg latanoprost ($P < 0.05$; Table 2). Similarly, a trend toward increased brain damage was seen in mice that were post-treated with 10 µg/kg latanoprost (94.2 ± 7.8 mm³), which became significant in the group that was given 100 µg/kg latanoprost (96.5 ± 7.7 mm³, $P < 0.05$) as compared to vehicle-treated WT mice (77.0 ± 15.0 mm³; Fig. 4). Interestingly, the deleterious effect of latanoprost was not observed in FP^{-/-} mice. In vehicle-treated groups, FP^{-/-} mice had significantly lower neurological deficit scores ($P < 0.05$; Table 2), and smaller infarct volumes than did the WT mice (38.2 ± 6.3 vs. 77.0 ± 15.0 mm³, $P < 0.05$). However, no significant difference in neurological deficit or brain infarct volume (39.2 ± 9.5 vs. 38.2 ± 6.3 mm³) was observed between latanoprost-treated FP^{-/-} and vehicle-treated FP^{-/-} mice.

Discussion

This study was specifically designed to investigate the effect of the FP receptor on neurobehavioral outcomes and infarct volume in a transient ischemia model of stroke in mice. Another set of experiments was conducted to further determine the role of FP in NMDA-induced excitotoxicity. We found that the resulting infarct volume after I/R injury was significantly smaller in mice lacking the FP receptor than in WT mice, while other physiological parameters monitored were unaffected. Unilateral induction of excitotoxicity also caused a significantly smaller lesion volume in FP^{-/-} than in WT mice. Finally, post-treatment of WT mice with the FP agonist latanoprost 30 min after the start of reperfusion significantly increased the neurological deficit and brain injury size as compared with those of the vehicle-treated control group; in contrast, latanoprost had no significant effect on the outcomes in the FP^{-/-} mice. The results suggest that FP receptors significantly contribute to exacerbation of excitotoxic and transient ischemic brain damage.

Recently, reports have suggested that COX-2-generated PGs perform diverse functions in numerous neurodegenerative disorders through their G-protein-coupled receptors (Ueno et al. 1982; Doré et al. 2003; Ahmad et al. 2005; Echeverria et al. 2005; Liu et al. 2005; Ahmad et al. 2006c; Kawano et al. 2006). Activation of the FP receptor initiates several

events, including the stimulation of the phospholipase C/inositol-3-phosphate/ Ca^{2+} signaling pathway (Heaslip and Sickels 1989; Abramovitz et al. 1994). Another study showed that FP receptor activation by $\text{PGF}_{2\alpha}$ in rodent cardiomyocytes caused protein kinase C-dependent activation of MAP kinase, which can also lead to increased expression of early growth response factor-1 (Xu et al. 2007). However, the mechanisms by which this receptor influences its signaling cascade in neurological conditions are not fully understood. We also observed that the amino acid sequence of the FP receptor has high sequence homology with that of the EP1 receptor. Moreover, on the phylogenetic tree of prostanoids, the FP receptor shares the same branch with the EP1 and TP receptors, as originally demonstrated by Narumiya's group (Toh et al. 1995). In addition, FP receptor mRNA is substantially expressed in rat whole brain (Kitanaka et al. 1994), and specific signals have been reported in C57BL/6 mouse brain (www.brain-map.org). Interestingly, we have previously shown that the EP1 receptor in C57BL/6 mice augments brain damage after excitotoxicity and focal cerebral ischemia (Ahmad et al. 2006a). Based on its similarity with the EP1 receptor in terms of evolution and amino acid sequence, we hypothesized that the FP receptor might contribute to excitotoxic and ischemic neuronal cell death similar to that caused by the EP1 receptor. Therefore, here we sought to determine the role of the FP receptor in transient focal cerebral ischemia and NMDA-induced excitotoxicity, and the results indicate that genetic deletion of the FP receptor results in a significant decrease in brain ischemic and excitotoxic damage in mice.

Studies have suggested that hypothermia and hyperthermia play important roles in outcome after cerebral ischemia (Dietrich and Bramlett 2007). Nonogaki and co-workers (Nonogaki et al. 1991) showed that hyperthermia is induced after injection of $\text{PGF}_{2\alpha}$ into the brains of rats. Therefore, to minimize variation in stroke outcomes in our experimental conditions, we closely monitored and maintained the rectal temperature of the mice. Our results indicate that we successfully minimized variation in rectal temperature, indicating that the differences in brain infarction that we observed between groups were unaffected by temperature differences.

The maintenance of physiological parameters, i.e., CBF, blood gases, and MABP, substantially influences the outcome of brain injury (Hackam and Spence 2007). Moreover, PGs are also likely mediators of vasomotor responses to hypoxia, ischemia, and hypertension (Hoffman et al. 1982; Chemtob et al. 1990a; Brault et al. 2003; Knock et al. 2005). The vasomotor response can also cause the release of PGE_2 , PGI_2 , and $\text{PGF}_{2\alpha}$ in the central nervous system (Chemtob et al. 1990a). It has been suggested that PGD_2 and PGI_2 are vasodilators. However, $\text{PGF}_{2\alpha}$ and TXA_2 are most likely constrictors, and the effects of PGE_2 depend on the tissue tested and the subtype of receptors being stimulated (Ellis et al. 1979; Kiriya et al. 1997). Under our experimental conditions, the MABP and CBF (as monitored by laser-Doppler flowmetry) were not significantly altered in WT or $\text{FP}^{-/-}$ mice, suggesting that at basal levels, the FP receptor did not significantly affect vasoregulation and that the attenuated brain damage in $\text{FP}^{-/-}$ mice was due mostly to the absence of a direct toxic effect of FP receptors.

The NMDA receptor is important for normal neurotransmission and physiological processes. However, activation of this receptor by agonists such as NMDA or glutamate results in

hyperactivity and leads to a pathological condition known as excitotoxicity (Choi 1992; Ayata et al. 1997; Iadecola et al. 2001; Lynch and Guttman 2002). Excitotoxicity is a leading factor among many that influence the pathogenesis of cerebral ischemia (Ogawa et al. 2007). Excitatory neurotransmitters appear to cause prolonged neuronal depolarization and trigger deregulation of cellular ion homeostasis, mainly through intracellular Ca^{2+} and Na^{+} . Influx of Ca^{2+} can lead to lipolysis, generation of free fatty acids, formation of reactive oxygen species, and finally to cell death (Bano and Nicotera 2007). We hypothesize that the attenuated infarction in the $\text{FP}^{-/-}$ mice is due in part to the absence of FP receptor excitotoxic effects. To test whether the FP receptor aggravates ischemic brain damage via excitotoxicity, we gave acute unilateral stereotactic injections of NMDA to WT and $\text{FP}^{-/-}$ mice. The resulting brain damage was less severe in $\text{FP}^{-/-}$ mice than in WT mice, a finding which supports our hypothesis that FP receptor activation potentially accentuates ischemic brain damage through excitotoxicity. This study is also supported by the findings of Lerea and co-workers (Lerea et al. 1997), who showed that NMDA causes a robust increase in the intracellular level of Ca^{2+} and release of arachidonic acid, which is metabolized to prostanooids by COX-2. Our study is further strengthened by a recent report showing that $\text{PGF}_{2\alpha}$ increases hypoxic neuronal injury in neuron-enriched primary cultures derived from rats (Li et al. 2008). The authors showed that addition of $\text{PGF}_{2\alpha}$ into the neuronal culture medium initiated significant cell death. They proposed that the generation of $\text{PGF}_{2\alpha}$ as a result of NMDA-induced COX-2 activity leads to an increase in the intracellular level of Ca^{2+} and subsequently to cell death.

Finally, we also found that pharmacological activation of the FP receptor by the agonist latanoprost increased neurological deficits and brain infarct size after MCAO. This result is in accordance with other studies that have shown that $\text{PGF}_{2\alpha}$ and latanoprost, by stimulating the FP receptor and initiating a downstream signaling cascade, lead to membrane depolarization and cell death (Minegishi et al. 2002; Cuppoletti et al. 2007). In addition, we tested the selectivity of latanoprost toward the FP receptor in $\text{FP}^{-/-}$ mice and found that the drug failed to aggravate brain damage after MCAO in those animals.

In summary, this study suggests that FP receptor activation aggravates acute excitotoxicity and I/R brain injury. Genetic deletion of the FP receptor results in significant attenuation of brain damage without significantly altering specific physiological parameters. Ongoing studies using receptor autoradiography are being pursued to determine the affinity and expression profiles of the FP receptor in mouse brain following various types of insult and to resolve the cellular and molecular mechanisms of action involved in the FP receptor-related neurotoxic effects. Our results provide a better understanding of the unique properties and functions of FP receptors in the injured brain and could lead to potential alternative therapies desperately needed for patients who suffer from acute or chronic neurological impairment.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health NS046400 and AG022971 (SD). We thank all members of the Doré lab team for assistance in this project, and Claire Levine for assistance in the preparation of the manuscript.

Abbreviations

CBF	Cerebral blood flow
COX	Cyclooxygenase
DMSO	Dimethyl sulfoxide
I/R	Ischemia-reperfusion
MABP	Mean arterial blood pressure
MCA	Middle cerebral artery
MCAO	Middle cerebral artery occlusion
PG	Prostaglandin
WT	Wild type

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Fig. 1.

FP receptor deletion does not affect physiological parameters. **a** Relative cerebral blood flow (CBF), **b** core body temperature, and **c** mean arterial blood pressure (MABP) were recorded at baseline, at induction of ischemia, and at 15-min intervals during ischemia and 1 h of reperfusion in WT and FP^{-/-} mice ($n = 5/\text{group}$). Change in CBF was recorded as a percent of baseline. Data are shown as mean $\pm$ SEM

Fig. 2. Effect of FP receptor deletion on neurological score and infarct volume. Mice were subjected to 90-min MCAO and tested for neurological deficit at 96 h. After the testing, mice were sacrificed and brain infarction was estimated by TTC staining. **a** Representative photographs show the infarcted brain slices from WT (*left*) and FP^{-/-} (*right*) mice. **b** Histogram shows the corrected hemispheric infarct volume of WT and FP^{-/-} mice. The infarct size (shown as mean ± SEM) was significantly smaller in FP^{-/-} than in WT mice; * $P < 0.05$. The neurological score deficit is represented in Table 2

Fig. 3.

FP receptor knockout decreases NMDA-induced neurotoxicity. WT ($n = 7$) and FP^{-/-} ($n = 7$) mice were injected stereotactically in the striatum with 15 nmol NMDA and sacrificed after 48 h. Brain sections were stained with Cresyl violet and analyzed for lesions. **a** Representative photographs of coronal sections from the brains of WT (*left panel*) and FP^{-/-} (*right panel*) mice after intrastratial injection with 15 nmol NMDA. The brain sections from the FP^{-/-} mouse show attenuation in lesion volume. **b** Histograms show that the FP^{-/-} mice were less vulnerable to the NMDA-induced neurotoxicity than were the WT mice. Values are reported as means $\pm$ SEM; * $P < 0.05$, when compared with WT group

Fig. 4.

Post-treatment of mice with the FP agonist latanoprost aggravates neurological deficit and brain infarction. Mice were subjected to MCAO for 90 min and then divided into five groups: WT + vehicle ($n = 6$), WT + 10 µg/kg latanoprost ($n = 10$), WT + 100 µg/kg latanoprost ($n = 8$), FP^{-/-} + vehicle ($n = 7$) and FP^{-/-} + 100 µg/kg latanoprost ($n = 7$). Injections of vehicle or latanoprost were given at 30 min of reperfusion. Neurological deficit was determined at 96 h after injection, before the mice were sacrificed, as represented in Table 2. **a** Representative photographs of coronal sections show brain infarction in (*left to right*) WT mice treated with vehicle, 10 µg/kg latanoprost, or 100 µg/kg latanoprost, and FP^{-/-} mice treated with vehicle or 100 µg/kg latanoprost. **b** Latanoprost-treated WT mice had significantly larger infarct volumes than did the vehicle-treated mice, whereas no effect was observed in FP^{-/-} mice. Values are shown as mean $\pm$ SEM. * $P < 0.05$ compared with the vehicle-treated WT control; # $P < 0.05$ compared with 100 µg/kg latanoprost-treated WT mice

Table 1Effect of MCAO on physiological parameters in WT and $FP^{-/-}$ mice

Parameter	WT mice		$FP^{-/-}$ mice		
	Baseline	1 h MCAO	1 h Reperfusion	Baseline	1 h MCAO 1 h Reperfusion
PH	7.34 $\pm$ 0.02	7.33 $\pm$ 0.01	7.34 $\pm$ 0.01	7.34 $\pm$ 0.01	7.33 $\pm$ 0.02 7.32 $\pm$ 0.04
PaCO ₂	38.4 $\pm$ 1.1	40.4 $\pm$ 1.2	38.9 $\pm$ 1.1	39.0 $\pm$ 1.1	40.3 $\pm$ 1.0 40.8 $\pm$ 1.8
PaO ₂	104 $\pm$ 3	130 $\pm$ 3	106 $\pm$ 2	114 $\pm$ 4	120 $\pm$ 3 113 $\pm$ 8

Table 2Effect of MCAO on neurological deficit score in WT and FP^{-/-} mice, and latanoprost-treated mice

Groups	Number of mice	Neurological deficit score			
		1	2	3	4
WT vs. FP ^{-/-}					
WT	9	22.2% (2/9)	77.8% (7/9)	0.0% (0/9)	0.0% (0/9)
FP ^{-/-} *	12	66.7% (8/12)	33.3% (4/12)	0.0% (0/12)	0.0% (0/12)
Latanoprost treatment					
WT + Vehicle	6	16.7% (1/6)	83.3% (5/6)	0.0% (0/6)	0.0% (0/6)
WT + Latano-10	10	10.0% (1/10)	60.0% (6/10)	30.0% (3/10)	0.0% (0/10)
WT + Latano-100 [†]	8	0.0% (0/8)	50.0% (4/8)	50.0% (4/8)	0.0% (0/8)
FP ^{-/-} + Vehicle [#]	7	71.4% (5/7)	28.6% (2/7)	0.0% (0/7)	0.0% (0/7)
FP ^{-/-} + Latano-100 ^{ns}	7	57.1% (4/7)	42.9% (3/7)	0.0% (0/7)	0.0% (0/7)

The distribution of neurological deficit scores were compared across the treatment groups using Fischer exact test.

* $P < 0.05$ when compared with the WT mice.[†] $P < 0.05$ when compared with the vehicle-treated WT group.[#] $P < 0.05$ when compared with the vehicle-treated WT group, *ns* (non-significant) when compared with the vehicle-treated FP^{-/-} group