HHS Public Access

Author manuscript

Neuroscience. Author manuscript; available in PMC 2018 June 20.

Published in final edited form as:

Neuroscience. 2010 September 29; 170(1): 317–323. doi:10.1016/j.neuroscience.2010.06.060.

NEUROPROTECTIVE PROPERTIES OF PROSTAGLANDIN I_2 IP RECEPTOR IN FOCAL CEREBRAL ISCHEMIA

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Abstract

We and others have identified that inhibition of cyclooxygenase might not be the optimal approach to limiting brain damage after stroke. Now we are investigating the unique properties of the various prostaglandin receptors to determine whether blocking those that mediate toxicity or stimulating those that reduce toxicity will improve neurological outcomes. Here, we determined the respective contribution of the prostaglandin I_2 (PGI_2) receptor in transient middle cerebral artery (MCA) occlusion (*t*MCAO) and permanent MCAO (*p*MCAO) preclinical stroke models by using male wildtype (WT) and IP receptor knockout ($IP^{-/-}$) C57Bl/6 mice. In addition, we investigated the putative preventive and therapeutic effects of the IP receptor agonist beraprost. The infarct volumes and neurological deficit scores (NDS) were significantly greater in $IP^{-/-}$ than in WT mice after both *t*MCAO and *p*MCAO. Interestingly, beraprost pretreatment (50 or 100 μg/kg p.o.) 30 min before *t*MCAO and post-treatment (100 μg/kg p.o.) at 2 or 4.5 h of reperfusion significantly reduced the neurological deficit score and infarct volume in WT mice. Post-treatment with beraprost (100 μg/kg p.o.) 4.5 h after *p*MCAO also significantly decreased neurological deficits and infarct volume in WT mice. Together, these novel findings suggest for the first time that PGI_2 IP receptor activation can attenuate anatomical and functional damage following ischemic stroke.

Keywords

beraprost sodium; cerebral ischemia; middle cerebral artery occlusion; mouse; prostacyclin

In ischemic stroke, multiple pathophysiological mechanisms promote brain injury through glutamate-mediated excitotoxicity, lipid peroxidation, peri-infarct depolarization, inflammation, and apoptosis (Traystman et al., 1991). Disturbances in hemostasis and increased inflammatory activity could contribute to and/or exacerbate cerebrovascular disease such as stroke (Brady et al., 2006). It has been reported that both cyclooxygenase (COX)-1 and COX-2 play an important role in inflammation, but their responses vary.

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COX-2 is highly inducible by inflammatory stimuli and increases the production of prostanoids (Teismann and Ferger, 2001; Lin et al., 2002; Doré et al., 2003; Narumiya, 2009). The prostanoids, generated through the combined actions of COX and synthetase enzymes, are divided into five main types: prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2. These prostanoids mediate their actions mainly through G-protein-coupled receptors: DP, EP, FP, IP, and TP. Each of these receptors differs in their respective effects on cyclic AMP (cAMP), phosphatidylinositol turnover, and intracellular calcium mobilization (Ahmad et al., 2006; Doré, 2006; Saleem et al., 2009).

PGI_2, also known as prostacyclin, is derived from arachidonic acid via the action of prostaglandin I_2 (PGI_2) synthetase and acts mainly on the membrane-bound IP receptors (Fang et al., 2006). It is an endogenous vascular regulator that acts mainly as a vasodilator (Moncada, 1982; Santhanam et al., 2010). PGI_2 analogs also appear to be potentially useful for the prevention of ischemia/reperfusion brain injury in gerbils and hypertensive rats (Kainoh et al., 1993; Matsuda et al., 1997). Previous reports have suggested that the IP receptor might provide protection by decreasing the neuronal cell death in traumatic brain injury and global cerebral ischemia (Lundblad et al., 2008; Wei et al., 2008). In support of the premise that IP is protective, IP receptor knockout ($IP^{-/-}$) mice were shown to develop more severe myocardial ischemic injury than their wildtype (WT) counterparts (Schror, 1987; Birnbaum et al., 2005).

In this study, we investigated the role of the IP receptor by measuring neurological deficits and infarct volumes in WT and $IP^{-/-}$ mice subjected to transient and permanent middle cerebral artery occlusion (MCAO) stroke models. In addition, we evaluated the putative neuroprotective properties of beraprost sodium, an orally active PGI_2 analog, in focal cerebral ischemia.

EXPERIMENTAL PROCEDURES

This study was performed in accordance with the NIH guidelines for the use of experimental animals. All protocols were approved by the Johns Hopkins Animal Care and Use Committee. WT and $IP^{-/-}$ C57BL/6 mice were maintained in our colonies and genotyped by polymerase chain reaction.

Transient focal cerebral ischemia (*t*MCAO)

Transient focal cerebral ischemia was induced by occluding the middle cerebral artery (MCA) with an intraluminal filament technique. Briefly, adult male mice (20–28 g; n=9 WT and 8 $IP^{-/-}$) were placed under halothane (Nicholas Piramal India Limited, Chennai, India) anesthesia. Body temperature was maintained at 37.0±0.5 °C. Relative cerebral blood flow (CBF) was monitored by laser-Doppler flowmetry (Moor Instruments, Devon, England), also called laser-Doppler flow (LDF) signal, over the parietal cortex supplied by the MCA. A 7-0 Ethilon nylon monofilament (Ethicon, Somerville, NJ, USA) coated with flexible silicone was used to occlude the MCA; successful occlusion was confirmed by a decrease in CBF. During the 90-min occlusion, anesthesia was discontinued, and the animals were housed in a humidity- and temperature-controlled chamber. Then the mice were briefly anesthetized, the filament was withdrawn, and mice were returned to the chamber for 6 h.

Then they were transferred to their respective cages and kept alive for 4 days. The investigator was not aware of the treatment group tested for neurological deficits before the mice were sacrificed for infarct measurement. Neurological function was graded on the following scale: 0=no deficit; 1=forelimb weakness and torso turning to the ipsilateral side when held by the tail; 2=circling to the affected side; 3=unable to bear weight on the affected side; and 4=no spontaneous locomotor activity or barrel rolling (Shah et al., 2006; Zeynalov et al., 2009).

Measurement of body temperature, blood gases, and mean arterial blood pressure

Body temperature was determined with a rectal probe in a separate cohort of animals (n=5) at baseline and at 15-min intervals for 90 min of ischemia and 60 min of reperfusion. The femoral artery was cannulated for measurement of arterial blood gases and mean arterial blood pressure (MABP), which were measured at the same time points.

Assessment of brain water content

In another cohort of mice (n=5/genotype), brain water content was measured by the wet/dry weight method, as described previously (Wang and Doré, 2007; Saleem et al., 2009). Mice were deeply anesthetized with halothane and decapitated to remove their brains. Samples were taken from ischemic and nonischemic hemispheres. The brains were weighed wet, oven dried at 100 °C for 48 h, and then reweighed. Percent brain water content was calculated as (wet weight − dry weight)/wet weight × 100.

Permanent focal ischemia (*p*MCAO)

The protocol used for *p*MCAO has been described previously (Saleem et al., 2009). Briefly, with the mice (n=9 WT and 8 $IP^{-/-}$) under halothane anesthesia, a 1.0-cm vertical skin incision was made between the right eye and ear. The temporal muscle was moved and the temporal bone exposed. Under a surgical microscope, a 2.0-mm burr hole was made just over the MCA, visible through the temporal bone. The main trunk of the distal part of the MCA was directly occluded with a bipolar coagulator, and complete interruption of blood flow at the occlusion site was confirmed by severance of the MCA occlusion site. Core body temperature was maintained between 36.5 and 37.5 °C during and after the procedures. Animals not circling toward the paretic side after the onset of ischemia and those that developed subarachnoid hemorrhage were eliminated from the study. A successful occlusion was also confirmed by placing the laser-Doppler probe above the temporal ridge to establish that blood flow into the region was terminated. After 7 days, the mice were euthanized and the brains harvested. To determine the neurological deficits caused by this model, an experimenter blinded to genotype tested all mice according to a robust 28-point scoring system (Saleem et al., 2009). The tests were divided into motor and sensory functions. For motor functions, the tests included were (1) spontaneous activity; (2) symmetry of walking; (3) head/neck movement when suspended by the tail; (4) symmetry of forelimbs when suspended by the tail; (5) climbing (45° angle); (6) balancing on a rod. For sensory function, the vibrissae test was used. Each test was graded from 1 to 4, establishing a maximum deficit score of 28. Immediately after the testing, the mice were sacrificed for infarct volume analysis, as described before (Saleem et al., 2009; Zeynalov et al., 2009).

Pretreatment and post-treatment with beraprost

Beraprost sodium is a stable, orally active PGI_2 analog with cyto-protective and anti-inflammatory properties (Melian and Goa, 2002). It is used for treatment and prevention of global cerebral ischemia in dogs and for cardiovascular diseases in humans (Kurihara et al., 1990; Murakami et al., 2005). In our study, one group of WT mice was pretreated with a single dose of vehicle (saline) or 50 or 100 μg/kg beraprost per os (p.o.) 30 min before induction of *t*MCAO (vehicle, *n*=8/group; 50 μg/kg beraprost, *n*=6/group; 100 μg/kg beraprost, *n*=11/group). Additionally, to determine the specificity of beraprost and confirm that its neuroprotective action is through the IP receptor, we pretreated $IP^{-/-}$ mice (*n*=6) with 100 μg/kg beraprost before subjecting them to the *t*MCAO model. Another group of WT mice was post-treated with 100 μg/kg beraprost (p.o.) at 2 or 4.5 h after the initiation of reperfusion (vehicle, *n*=7/group; 100 μg/kg beraprost (2 h), *n*=8/group; 100 μg/kg beraprost (4.5 h), *n*=8/group). A final group was post-treated with vehicle (*n*=9) or 100 μg/kg beraprost (*n*=5) 4.5 h after the initiation of *p*MCAO.

Quantification of infarct volume in both ischemic stroke models

After neurological assessment, the 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. Infarct area of each section on all of the slides was measured with Image Analysis Software (Sigma Scan Pro, Systat, Port Richmond, CA, USA). The investigator was not aware of the treatment groups. Infarct volume was converted by multiplying the measured infarct volume by the ratio of the contralateral structure to the ipsilateral structure (Zeynalov et al., 2009).

Statistical analysis

The data were analyzed by one-way ANOVA followed by New-man–Keuls multiple range test. Data are represented as mean±standard error of the mean (SEM). Neurological deficit scores (NDS) were analyzed by the nonparametric Kruskal–Wallis analysis of ranks. A *P*-value <0.05 was considered to be statistically significant.

RESULTS

Effect of IP receptor on physiological parameters

Table 1 shows the pH, $PaCO_2$, and PaO_2 of WT and $IP^{-/-}$ mice. No significant differences were observed in these parameters among the tested groups prior to MCAO, 1 h after MCAO, or 1 h after reperfusion. The LDF signal, body temperature, and MABP also did not differ between the two groups (Fig. 1). We have previously reported that there are no obvious differences in gross cerebrovascular anatomy between the WT and the $IP^{-/-}$ groups (Wei et al., 2008). In addition, no significant differences in physiological parameters were observed between mice administered the effective dose of beraprost (100 μg/kg) and those administered vehicle [MABP (90–80 mm Hg), temperature (37.0–37.5 °C), and pH (7.34–7.37)].

Genetic deletion of the IP receptor exacerbates neurological deficit scores and infarct volume in the *t*MCAO model

Four days after *t*MCAO, the estimated mortality rate in the $IP^{-/-}$ mice was 30% whereas in WT control mice, it was approximately 20%. Interestingly, the $IP^{-/-}$ mice had significantly greater neurological impairment ($P<0.01$) than did the WT mice as measured by NDS. Moreover, corrected infarct volume in $IP^{-/-}$ mice was significantly larger ($P<0.01$) than that in the WT mice (Fig. 2).

Effect of IP receptor on brain water content

To further substantiate our outcomes regarding the role of the IP receptor, we evaluated water content in each hemisphere of the WT and $IP^{-/-}$ mice (Fig. 3). When the contralateral hemispheres were compared, the water content of the $IP^{-/-}$ mice did not differ substantially from that of the WT mice. However, the mean water content of the ipsilateral hemispheres was significantly higher ($P<0.01$) in the $IP^{-/-}$ mice than in the WT mice, demonstrating that brain edema is accentuated in the $IP^{-/-}$ mice.

Effect of pretreatment with beraprost on neurological deficit scores and infarct volume in the *t*MCAO model

NDS and infarct volume were significantly reduced in WT mice pretreated with 50 μg/kg beraprost ($P<0.05$) and 100 μg/kg beraprost ($P<0.01$) as compared to values in the vehicle-treated mice. In contrast, pretreatment with 100 μg/kg beraprost had no effect in $IP^{-/-}$ mice (Fig. 4). To determine the therapeutic time window, WT mice were also post-treated with 100 μg/kg beraprost at 2 and 4.5 h after the start of cerebral reperfusion. Beraprost significantly reduced the NDS and corrected infarct volume at both time points (both $P<0.05$; Fig. 5).

Genetic deletion of the IP receptor exacerbates neurological deficit scores and infarct volume in the *p*MCAO model

Because the *p*MCAO model produces small cortical infarcts in mice and the survival rate is 100%, we used this model as in our previous studies to further determine the delayed ischemic effects in $IP^{-/-}$ mice. After 7 days of *p*MCAO, $IP^{-/-}$ mice had significantly higher NDS and larger infarct volumes (both $P<0.01$) than did WT mice following an identical experimental protocol (Fig. 6).

Effect of post-treatment with beraprost on neurological deficit scores and infarct volume in the *p*MCAO model

To determine whether post-treatment with beraprost could have a therapeutic effect in the permanent focal ischemic model, we administered 100 μg/kg of the drug 4.5 h after the initiation of *p*MCAO. On day 7, the NDS and cortical infarct volume were significantly reduced (both $P<0.01$) as compared to mice given vehicle (Fig. 7).

DISCUSSION

This study was designed to define a potential endogenous defense system that could counterbalance the adverse effects of focal cerebral ischemia. We and others have previously reported that some endogenous prostaglandins generated through COX-2 activity play a beneficial role against focal ischemia (Ahmad et al., 2006; Saleem et al., 2007). We have also reported that PGI_2 IP receptors could be beneficial in global ischemia (Wei et al., 2008), and here we investigated the importance of the IP receptor in two complementary models of focal ischemia (transient and permanent) by assessing the neurological deficit and brain damage in WT and $IP^{-/-}$ mice. In addition, we determined the preventive and therapeutic potential of the orally active selective IP receptor agonist beraprost in both MCAO models. Our results suggested for the first time that the neurological deficits and infarct volumes were greater in $IP^{-/-}$ mice than in WT mice in both the *t*MCAO and *p*MCAO stroke models. The brain water content was also attenuated in the $IP^{-/-}$ mice compared to that in WT controls. In addition, pre- and post-treatment with bera-prost significantly improved the negative outcomes of these stroke models as estimated by the NDS and infarct size in WT mice.

Over recent years, it has been publicized that the use of COX-2 inhibitors for conditions such as arthritic pain can cause adverse effects related to heart disease and stroke (Doré et al., 2003; Baigent and Patrono, 2008). Therefore, defining the function of receptors that lie downstream of prostanoid synthesis, such as the PGI_2 IP receptor, could provide useful targets for new, more selective therapeutic strategies. PGI_2 is most abundantly present in cerebral cortex, striatum, and hippocampus (Matsumura et al., 1995). Because of its properties as an anti-ischemic factor, PGI_2 possesses cerebrovascular and cardiovascular activities (Viinikka, 1984; Jeremy et al., 1987). It has been reported that deletion of the IP receptor increases susceptibility to cardiovascular abnormalities, thrombotic stimuli, and atherogenesis (Xiao et al., 2001; Francois et al., 2005). Conversely, stable PGI_2 analogs such as beraprost, which has anti-platelet, anti-thrombotic, and anti-inflammatory properties, protect against cerebral ischemia in hypertensive rats (Kainoh et al., 1993). Thus the stroke outcomes observed here might also be caused by a combination of physiological phenomena. What's more, the use of two different stroke paradigms, one that includes reperfusion and one that does not, enabled us to address the therapeutic effects of beraprost in different pathophysiological conditions.

Reports are conflicting as to whether or not PGI_2 improves the overall CBF (van den Kerckhoff et al., 1983). The laser-Doppler method of CBF measurement is most widely used and has the advantage of being able to depict temporal changes in CBF in real time. In our present study, we found no significant difference in CBF as estimated by LDF between the WT and $IP^{-/-}$ mice, though future work to fully investigate absolute blood flow and CBF regulation is warranted. Furthermore, no changes in MABP or blood gases were observed between the two genotypes.

It is well established that hypothermia or hyperthermia can affect ischemic stroke outcomes (Kurihara et al., 1990; Leira et al., 2006). Increased temperature enhances mortality and morbidity, whereas a reduction in brain and/or body temperature has a protective effect. A

few studies have suggested that PGI_2 might have a role in temperature regulation (Evora et al., 2007). Under our experimental design, no significant difference in body temperature between WT and $IP^{-/-}$ mice was observed during or after focal cerebral ischemia.

PGI_2 can also play an important role in brain edema in cerebral ischemia (Katayama et al., 1992). While changes in the brain water content can correlate with ischemic size, it is also well accepted that focal ischemia, particularly if it is followed by reperfusion, leads to damage of the blood-brain barrier and allows water and macromolecules to pass from the vessels into the tissue (Toung et al., 2002). It has also been reported previously that PGI_2 and its analog beraprost protect against brain edema (Pluta et al., 1990). Our results suggest that genetic deletion of the IP receptor exacerbates the increase in brain water content that occurs ipsilateral to MCAO.

It has been reported that activated IP receptors signal mainly through G_{as} activation of adenylyl cyclase and enhancement of cAMP concentrations (Sprague et al., 2008). It has also been suggested that PGI_2 and its analogs can reduce neuronal cell death by inhibiting excessive influx of calcium into neuronal cells (Pluta et al., 1990). Furthermore, recent reports indicate that the IP receptor and other prostanoid receptors, such as EP2, EP4, and DP1, which also activate G_{as}, have neuroprotective functions, mainly through the cAMP/protein kinase A signaling pathway (Wilson et al., 2004; Echeverria et al., 2005; Saleem et al., 2007). Future work will be required to investigate and fully appreciate the complexity of the intracellular pathways that lead to brain protection from ischemic damage. Taken together, the evidence suggests that the IP receptor likely protects neurons by enhancing intracellular cAMP production and reducing calcium overload in neurons to mitigate subsequent neuronal cell death following either focal or global stroke (Wei et al., 2008).

To our knowledge, this is the first study to investigate the role of the PGI_2 IP receptor and its pharmacologic agonist in mouse focal ischemic stroke models. We have shown that genetic deletion of the IP receptor exacerbates ischemic brain injury without affecting physiological parameters. We also demonstrated that pre- and post-treatment with the stable PGI_2 agonist beraprost can reduce the severity of stroke outcomes. Thus, the IP receptor might play a significant role in dictating stroke outcomes.

Acknowledgments

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. This work was supported in part by grants from the National Institutes of Health NS046400 and AG022971 (SD).

Abbreviations

cAMP	cyclic AMP
CBF	cerebral blood flow
COX	cyclooxygenase
LDF	laser-Doppler flow

MABP	mean arterial blood pressure
MCA	middle cerebral artery
NDS	neurological deficit scores
PGI_2	prostaglandin I_2
***p*MCAO**	permanent middle cerebral artery occlusion
***t*MCAO**	transient middle cerebral artery occlusion
WT	wildtype

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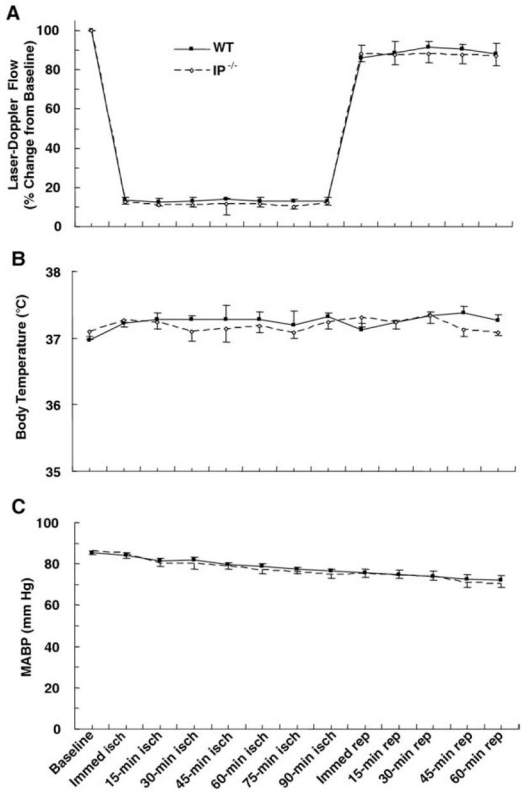

Fig. 1.

Absence of the IP receptor does not affect the monitored physiological parameters. Laser-Doppler flow (LDF) signal (A), core body temperature (B), and mean arterial blood pressure (MABP, C) were recorded at baseline, at induction of ischemia (immed), and at 15-min intervals during ischemia and 1 h of reperfusion. Change in LDF signal was recorded as a percent of baseline.

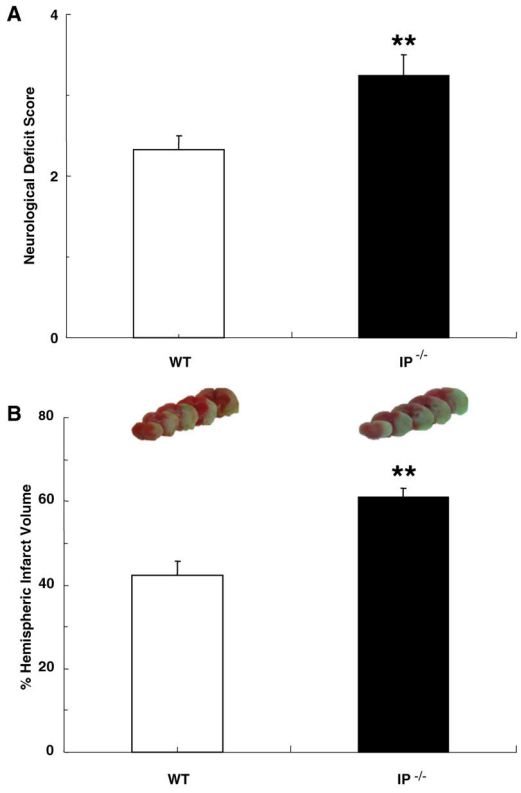

Fig. 2.

Deletion of IP enhances ischemic brain injury and neurological dysfunction after transient ischemia. WT and $IP^{-/-}$ mice were subjected to 90 min of ischemia and 4 d of reperfusion. (A) Neurological scores assessed 4 d after ischemia were significantly higher in $IP^{-/-}$ mice than in WT mice, indicating greater neurological dysfunction. (B) Photographs of infarcted brain slices from WT (left) and $IP^{-/-}$ (right) mice. Percent corrected hemispheric infarct volume was significantly larger in $IP^{-/-}$ mice than in WT mice after 4 d of reperfusion. ** $P<0.01$ vs. WT. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

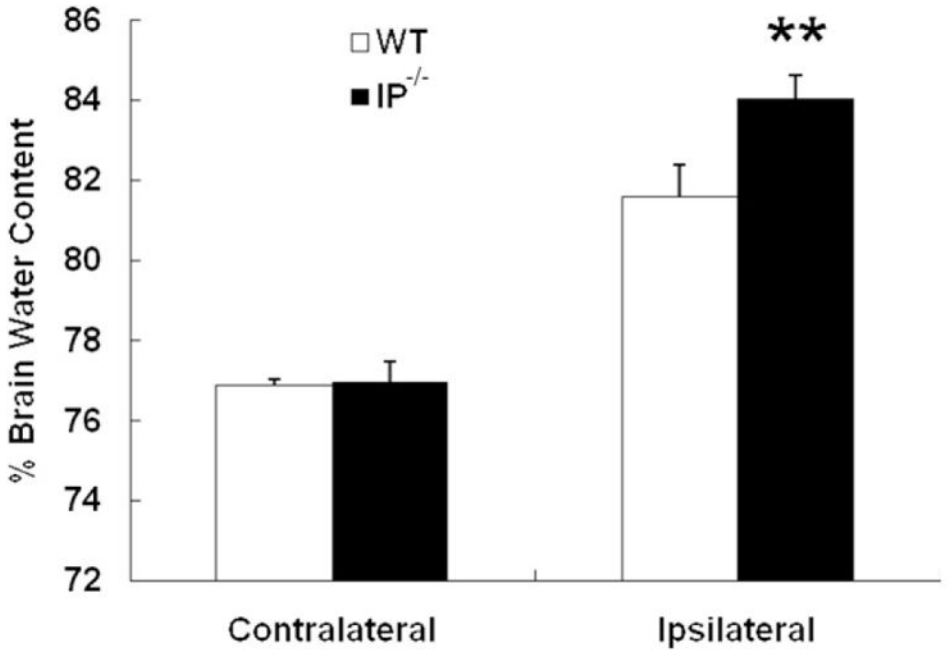

Fig. 3.

Water content in ischemic and nonischemic hemispheres of WT and $IP^{-/-}$ mice 4 d after transient middle cerebral artery occlusion. ** $P<0.01$ vs. WT.

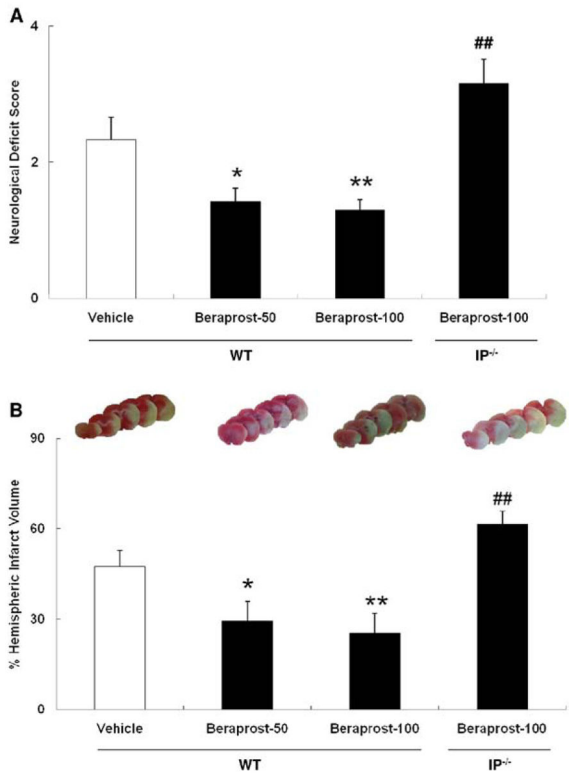

Fig. 4.

Pretreatment with beraprost reduces ischemic brain injury and neurological dysfunction after transient ischemia. WT mice were pre-treated with vehicle or beraprost and exposed to 90 min of ischemia and 4 d of reperfusion. (A) Neurological scores assessed 4 d after ischemia were significantly lower in WT mice pretreated with beraprost than in vehicle-treated mice, indicating lesser neurological dysfunction. (B) Left to right: photographs of infarcted brain slices from a vehicle-treated WT mouse, WT mouse pretreated with 50 μg/kg beraprost, WT mouse pretreated with 100 μg/kg beraprost, and $IP^{-/-}$ mouse pretreated with 100 μg/kg beraprost. Percent corrected hemispheric infarct volume was significantly smaller in beraprost-treated WT mice than in vehicle-treated WT mice after 4 d of reperfusion. Beraprost did not protect $IP^{-/-}$ mice from ischemic brain injury. * $P<0.05$; ** $P<0.01$ vs. vehicle; $^{\#\#}$ $P<0.01$ vs. WT+100 μg/kg beraprost. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

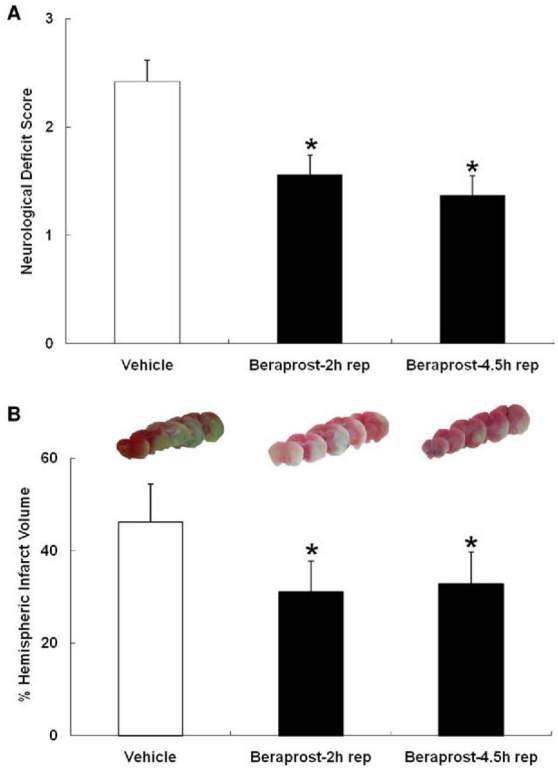

Fig. 5.

Post-treatment with beraprost reduces ischemic brain injury and neurological dysfunction after transient ischemia. WT mice were subjected to 90 min of ischemia and administered 100 μg/kg beraprost after 2 or 4.5 h of cerebral reperfusion (rep). (A) Neurological scores assessed 4 d after ischemia were significantly lower in beraprost-treated mice than in vehicle-treated mice, indicating lesser neurological dysfunction. (B) Photographs of infarcted brain slices from a vehicle-treated mouse (left) and mice post-treated with beraprost at 2 h (center) and 4.5 h (right). Percent corrected hemispheric infarct volume was significantly lower in beraprost-treated mice than in vehicle-treated mice after 4 d of reperfusion. * $P<0.05$ vs. vehicle. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

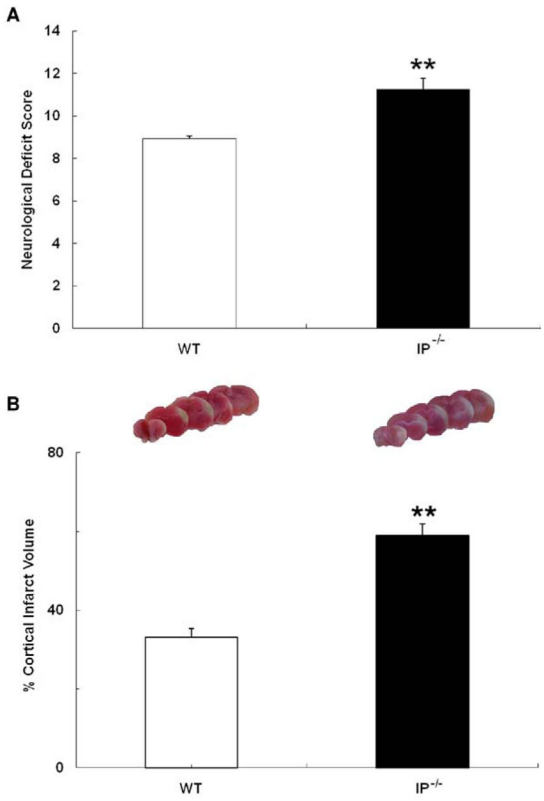

Fig. 6.

Deletion of IP receptor enhances ischemic brain injury and neurological dysfunction after permanent distal middle cerebral artery occlusion. (A) Neurological scores assessed after 7 d of ischemia were significantly higher in $IP^{-/-}$ mice than in WT mice, indicating greater neurological dysfunction. (B) Photographs of infarcted brain slices from WT (left) and $IP^{-/-}$ (right) mice. Percent corrected cortical infarct volume was significantly larger in $IP^{-/-}$ mice than in WT mice after 7 d of ischemia. ** $P<0.01$ vs. WT. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

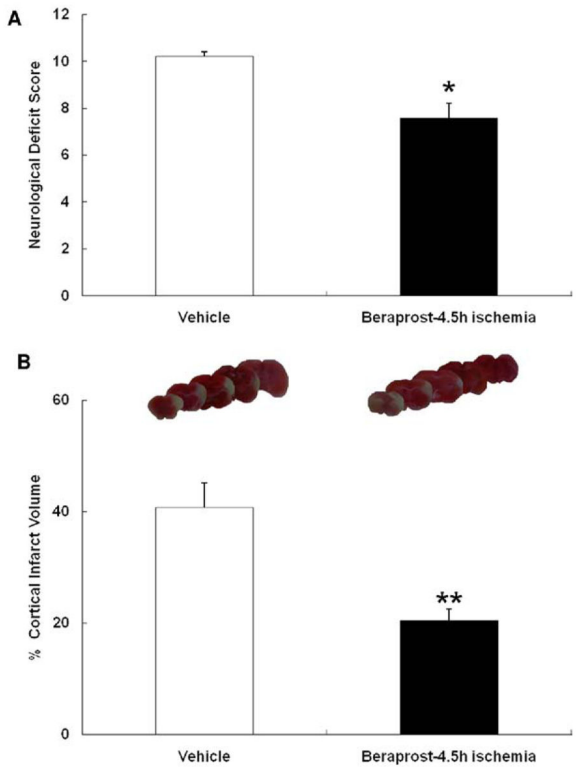

Fig. 7.

Post-treatment with beraprost reduces cortical infarct volume and neurological dysfunction after permanent ischemia. WT mice were administered 100 μg/kg beraprost 4.5 h after being subjected to permanent cerebral ischemia. (A) Neurological scores assessed after 7 d of ischemia were significantly lower in beraprost-treated mice than in vehicle-treated mice, indicating lesser neurological dysfunction. (B) Photographs of infarcted brain slices from vehicle-treated (left) and beraprost-treated (right) WT mice. Percent corrected cortical infarct volume was significantly decreased in beraprost-treated mice compared to vehicle-treated mice after 7 d. * $P<0.05$, ** $P<0.01$ vs. vehicle. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Table 1

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

Parameter	Wildtype mice			$IP^{-/-}$ mice		
	Baseline	1 h MCAO	1 h Reperfusion	Baseline	1 h MCAO	1 h Reperfusion
pH	7.34±0.01	7.33±0.01	7.33±0.02	7.34±0.01	7.33±0.01	7.32±0.01
$PaCO_2$	40.7±0.9	41.1±1.0	41.0±0.9	40.1±1.0	40.4±1.0	42.0±0.3
PaO_2	117±5	123±5	124±7	116±5	120±5	118±4

The values are expressed as mean±SEM (n=5/group).