Prostaglandin D₂ DP1 receptor is beneficial in ischemic stroke and in acute excitotoxicity in young and old mice

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Received: 21 July 2009 / Accepted: 29 January 2010 / Published online: 5 March 2010
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Abstract The cardiovascular complications reported to be associated with cyclooxygenase inhibitor use have shifted our focus toward prostaglandins and their respective receptors. Prostaglandin D₂ and its DP1 receptor have been implicated in various normal and pathologic conditions, but their role in stroke is still poorly defined. Here, we tested whether DP1 deletion aggravates *N*-methyl-D-aspartic acid (NMDA)-induced acute toxicity and whether DP1 pharmacologic activation protects mice from acute excitotoxicity and

transient cerebral ischemia. Moreover, since the elderly are more vulnerable to stroke-related damage than are younger patients, we tested the susceptibility of aged DP1 knockout (DP1^{-/-}) mice to brain damage. We found that intraatrial injection of 15 nmol NMDA caused significantly larger lesion volumes (27.2±6.4%) in young adult DP1^{-/-} mice than in their wild-type counterparts. Additionally, intracerebroventricular pretreatment of wild-type mice with 10, 25, and 50 nmol of the DP1-selective agonist BW245C significantly attenuated the NMDA-induced lesion size by 19.5±5.0%, 39.6±7.7%, and 28.9±7.0%, respectively. The lowest tested dose of BW245C also was able to reduce middle cerebral artery occlusion-induced brain infarction size significantly (21.0±5.7%). Interestingly, the aggravated NMDA-induced brain damage was persistent in older DP1^{-/-} mice as well. We conclude that the DP1 receptor plays an important role in attenuating brain damage and that selective targeting of this receptor could be considered as an adjunct therapeutic tool to minimize stroke damage.

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Keywords BW245C · G-protein-coupled receptors ·
Mouse · Neurodegeneration · Neuroprotection ·
NMDA · Prostaglandins

Introduction

Stroke is one of the leading causes of neurologic dysfunction and death but has limited therapeutic

options (Rosamond et al. 2008). Treatments that are available must be given within 3 h of onset. Delayed neuronal cell death follows ischemic–reperfusion injury mainly as a result of free radical damage and neuro-inflammatory cascade (Moore and Traystman 1994; Chan 2001; Endres et al. 2008). Cyclooxygenase (COX), a proinflammatory mediator, has been implicated in the progression of stroke damage. During various pathologic events, COX-2 is highly upregulated; therefore, COX-2 inhibitors have been considered to be potential therapeutic remedies against stroke. Unfortunately, clinical trials associate COX-2 inhibitors with enhanced risk of cardiovascular complications (Bresalier et al. 2005; Graham et al. 2005). Therefore, focus has shifted toward the cascade of prostaglandins downstream of COX enzymes.

Prostaglandin D₂ (PGD₂) is formed by the action of PGD synthases on the COX product PGH₂, and recently PGD synthases have been suggested to be protective in ischemic stroke (Saleem et al. 2009). PGD₂ is widely distributed in rat and human brain (Abdel-Halim et al. 1977; Narumiya et al. 1982; Ogorochi et al. 1984). In peripheral tissues, PGD₂ executes a wide range of functions, including vasodilatation, inhibition of platelet aggregation, glycogenolysis, vasoconstriction, allergic reaction mediation, and intraocular pressure reduction (Whittle et al. 1983; Narumiya and Toda 1985; Casteleijn et al. 1988; Sturzebecher et al. 1989; Darius et al. 1994; Matsugi et al. 1995; Matsuoka et al. 2000; Angeli et al. 2004). In the brain, PGD₂ has been shown to contribute to sleep induction, modulation of body temperature, olfactory function, hormone release, nociception, and neuromodulation (Eguchi et al. 1999; Urade and Hayaishi 1999; Mizoguchi et al. 2001; Hayaishi 2002; Hayaishi and Urade 2002; Gelir et al. 2005).

PGD₂ exerts its effects by binding with the membrane-bound G-protein-coupled DP1 receptor. The DP1 receptor is coupled to G_s protein and stimulates adenylyl cyclase, leading to increased levels of cyclic adenosine monophosphate (cAMP; Hata and Breyer 2004). A second type of DP receptor [DP2, also referred to as chemoattractant receptor-homologous molecule expressed on T helper cells (CRTH2)] has also been reported, but it has no significant homology with DP1 and has different biological functions and cellular distribution (Hirai et al. 2001; Sawyer et al. 2002), and some of its signaling is thought to be independent of PGD₂ levels. Furthermore, it has been

documented that its activation decreases cAMP and increases intracellular calcium levels (Bohm et al. 2004; Sandig et al. 2006). The *in vivo* role of DP1 in the peripheral nervous system has been well investigated (Angeli et al. 2004; Hata and Breyer 2004; Koch et al. 2005), but our knowledge of its role in the brain is still very limited (Campbell and Feinberg 1996; Urade and Hayaishi 1999; Hayaishi and Urade 2002; Obal and Krueger 2003).

Activation of DP1 receptors is primarily associated with anti-inflammatory effects, but proinflammatory effects of DP1 also have been described (Matsuoka et al. 2000; Hammad et al. 2003; Angeli et al. 2004; Spik et al. 2005). It has been reported that PGD₂ induces nerve growth factor and brain-derived neurotrophic factor (Toyomoto et al. 2004). In addition, a study by Andreasson's group showed that the DP1 agonist BW245C increases the level of cAMP and protects hippocampal slice cultures against *N*-methyl-D-aspartic acid (NMDA)-induced excitotoxicity (Liang et al. 2005). Our data previously revealed that mice lacking DP1 are more susceptible to middle cerebral artery occlusion (MCAO)-induced brain damage than are wild-type (WT) mice (Saleem et al. 2007). Because excitotoxicity is a major component in propagating stroke damage, here we hypothesized that genetic deletion of the DP1 receptor would also augment NMDA-induced neurotoxicity and that activation of the DP1 receptor would attenuate the brain damage caused by excitotoxicity and MCAO. We also tested whether the NMDA-induced brain damage augmentation would be sustained in aged DP1^{−/−} mice. To test this hypothesis, we compared the outcomes in young and old WT and DP1^{−/−} mice subjected to an NMDA model of acute excitotoxicity. In addition, the pharmacological effect of the DP1 agonist BW245C on outcomes from NMDA-induced excitotoxicity and MCAO in young adult male WT mice was also determined. To the best of our knowledge, this is the first study to test the *in vivo* effect of BW245C against acute excitotoxicity and MCAO.

Materials and methods

Animals and drugs

Studies were carried out on 8–10-week-old male C57BL/6 mice (20–25 g) obtained from Charles River

Laboratories, Inc. (Wilmington, MA, USA). To determine the effect of the DP1 receptor in aged mice, 14–18-month-old WT and DP1^{-/-} mice were used. Colonies of WT and DP1^{-/-} mice (obtained from Dr. Shuh Narumiya (Matsuoka et al. 2000)) were maintained in our animal facility. These mice were back-crossed eight to ten generations, and genotype was determined by PCR before the experiment. The DP1^{-/-} mice develop normally and have no gross abnormalities in behavior, macroscopic anatomy, or biochemical or hematologic indices (Matsuoka et al. 2000). All animal protocols were approved by the Johns Hopkins University Animal Care and Use Committee. The animals were allowed free access to water and food before and after surgery. BW245C [5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin] was purchased from Cayman Chemicals, Ann Arbor, MI, USA. BW245C is a selective DP1 agonist with high binding affinity (K_i =250 nM) toward mouse DP1 (Kiriya et al. 1997; Narumiya et al. 1999); it has been shown to increase intracellular levels of cAMP and activate adenylyl cyclase (Ientile et al. 1983). Earlier studies proposed that BW245C had nonspecific effects through EP2 and EP4 receptors (Giles et al. 1989; Hamid-Bloomfield and Whittle 1989; Matsugi et al. 1995; Rangachari et al. 1995); however, these effects were reported to be species specific. Nevertheless, nonspecific effects of BW245C in neurological settings have not been reported in mice. The known affinity of BW245C toward mouse DP1 is more than seven times its affinity toward FP and more than 40 times its affinity toward other mouse prostanoid receptors. Correspondingly, the affinity of BW245C toward human DP1 is approximately 330 times higher than toward human EP4 and 547 times higher than toward human EP2 (Kiriya et al. 1997; Wright et al. 1998; Abramovitz et al. 2000; Narumiya and FitzGerald 2001).

Pretreatment with BW245C

After their weight and rectal temperature were recorded, mice were anesthetized and placed on a stereotaxic stand for intracerebral microinjections as described previously (Ahmad et al. 2006a). Briefly, mice were given a single 0.2- μ l injection containing 10 nmol ($n=9$), 25 nmol ($n=7$), or 50 nmol ($n=6$) BW245C (for excitotoxicity model) or 10 nmol BW245C (for MCAO model; $n=8$) in the right cerebral ventricle. After the injection, the hole was

plugged with bone wax, and the skin overlying the skull was sutured. Mice were then prepared for either stereotaxic NMDA injection or the MCAO procedure.

Unilateral NMDA-induced acute excitotoxicity model

Twenty minutes after the BW245C treatment, 15 nmol NMDA in a volume of 0.3 μ l was injected slowly into the right striatum; 5 min later, the needle was retracted slowly, the hole was blocked with bone wax, and the skin was sutured. Two control groups were used. One received only the 15-nmol intrastriatal NMDA injection ($n=7$), whereas the other received a 0.2- μ l intracerebroventricular (ICV) injection of vehicle (0.5% DMSO) 20 min before the NMDA injection ($n=6$). To test the effect of NMDA-induced toxicity in young and old DP1^{-/-} mice, 8–10-week-old WT ($n=7$) and DP1^{-/-} ($n=6$) mice and 14–18-month-old WT ($n=8$) and DP1^{-/-} ($n=8$) mice were given a single intrastriatal injection of NMDA. After the procedure, mice were placed in a thermoregulated chamber to recover from anesthesia before being transferred to their home cages and allowed to survive for 48 h. Throughout the experimental procedure, rectal temperature of mice was monitored and maintained at $37.0\pm 0.5^\circ\text{C}$.

Quantification of the excitotoxic lesion volume

At 48 h after NMDA injection, weight and rectal temperature were recorded, and mice were deeply anesthetized with pentobarbital and transcardially perfused with cold PBS followed by 4% paraformaldehyde in PBS (pH 7.2). Brains were harvested quickly, postfixed for 24 h, and then equilibrated in 30% sucrose and frozen in precooled 2-methylbutane. Sequential brain sections of 25 μ m obtained on a cryostat were stained with Cresyl Violet, and lesion volume was analyzed according to the protocol described previously (Ahmad et al. 2006b).

MCAO and reperfusion

During the MCAO procedure, mouse rectal temperature was monitored and maintained at $37.0\pm 0.5^\circ\text{C}$ by a heating pad, and anesthesia was maintained with continuous flow of halothane in oxygen-enriched air via a nose cone. Relative cerebral blood flow was monitored with laser Doppler flowmetry (Moor

Instruments, Devon, England) by a flexible fiber-optic probe affixed to the skull over the parietal cortex supplied by the middle cerebral artery (2 mm posterior and 6 mm lateral to the bregma). MCAO was carried out under aseptic conditions with a silicone-coated nylon monofilament as described previously (Ahmad et al. 2006c). The filament was left in position for 90 min, during which the incision was closed with sutures, anesthesia was discontinued, and the animals were transferred to a temperature-controlled chamber to maintain their body temperature at $37.0 \pm 0.5^\circ\text{C}$. At 90 min of occlusion, the mice were briefly reanesthetized with halothane, and reperfusion was achieved by withdrawing the filament. After their incision was sutured, the mice were returned to the temperature-controlled chamber for 2 h before being transferred to their home cages for 4 days.

Analysis of physiologic parameters

Physiologic parameters were measured in a group of mice separate from that used to assess infarct volume. Mice were injected with either vehicle ($n=6$) or 10 nmol BW245C ($n=6$) before being subjected to MCAO. Arterial blood samples collected via femoral catheter were analyzed for pH, PaO_2 , and PaCO_2 before the occlusion, during occlusion, and 1 h after the occlusion. Mean arterial blood pressure (MABP) was measured at the same time points by a pressure transducer connected to the femoral catheter.

Temperature regulation

A third cohort of mice was implanted with intra-abdominal radiofrequency probes [IPTT-200, Bio Medic Data System (BMDS), Seaford, DE] 7 days before injection of BW245C or vehicle ($n=6$ per group). Core temperature was sampled at 10-min intervals from before the mice were anesthetized until 90 min after injection and then once daily for 4 days at room temperature via receivers (DAS-5002 Notebook System™; BMDS). This telemetry system minimizes stress and allows temperature control and monitoring in freely moving animals.

Quantification of infarct volume

Four days after surgery, mice were deeply anesthetized, and their brains were harvested and sliced

coronally into 2-mm-thick sections. The sections were incubated with 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) in saline for 20 min at 37°C . The area of infarcted brain, 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 in each slice (SigmaScan Pro, SPSS, Port Richmond, CA, USA). Volumes from all five slices were summed to calculate total infarct volume over the entire hemisphere and expressed as a percentage of the volume of the contralateral structure. Infarct volume was corrected for swelling by comparing the volumes in the ipsilateral and contralateral hemispheres. The corrected infarct volume was calculated as: volume of contralateral hemisphere – (volume of ipsilateral hemisphere – volume of infarct).

Statistical analysis

The brain sections were imaged and analyzed with SigmaScan Pro 5.0 software (Systat, Inc., Point Richmond, CA, USA). Statistical analysis was performed by one-way ANOVA followed by Bonferroni multiple comparison test; P values <0.05 were considered to be significant. All values are expressed as mean $\pm$ standard error of the mean (SEM).

Results

Genetic deletion of DP1 receptor aggravates excitotoxic brain damage

$\text{DP1}^{-/-}$ mice were found to be more vulnerable to NMDA-induced toxicity as compared to the WT mice (Fig. 1a). Quantification showed that NMDA-treated $\text{DP1}^{-/-}$ mice had a mean lesion volume of 8.29 mm^3 , which was significantly greater than that of the WT mice, which had a mean lesion volume of 6.51 mm^3 ($P<0.05$; Fig. 1b).

NMDA-induced brain damage is decreased by BW245C

NMDA-induced brain damage in the ipsilateral striatum appeared to be attenuated by pretreatment with BW245C (Fig. 2a). Analysis of the stained sections revealed a significant decrease in the lesion

Fig. 1 NMDA-induced toxicity is aggravated in $DP1^{-/-}$ mice. A single dose of 15 nmol NMDA was injected stereotactically into the striatum of C57BL/6 WT ($n=7$) and $DP1^{-/-}$ mice ($n=6$). **a** Representative photographs of coronal sections of the brains of WT (left panel) and $DP1^{-/-}$ (right panel) mice show aggravated lesion in the latter. **b** Analysis of the Cresyl-Violet-stained brain sections shows that the NMDA-induced lesion size was significantly greater in the $DP1^{-/-}$ mice. $*P<0.05$ when compared with the WT group

volume of BW245C-pretreated groups compared with the vehicle-treated control group (Fig. 2b). The lesion volume decreased significantly ($P<0.05$) by $19.5\pm 5.0\%$, $39.6\pm 7.6\%$, and $28.9\pm 7.0\%$ in the 10-, 25-, and 50-nmol-treated groups, respectively. Pretreatment with vehicle did not affect the level of NMDA-induced toxicity.

BW245C attenuates infarct volume in WT mice

We chose the lowest dose of BW245C that was effective in the NMDA model to determine whether it had the potential to minimize MCAO outcome. We found that mice given an ICV injection of 10 nmol BW245C immediately before MCAO had hemispheric infarct volumes that were $21.0\pm 5.7\%$ ($P<0.05$) smaller than those of the vehicle-treated group (Fig. 3).

BW245C does not affect physiologic parameters

The physiologic parameters that we measured (pH, $PaCO_2$, PaO_2 , and MABP) were within normal physiologic ranges for all mice, and none showed any noteworthy differences between the vehicle-

treated group and the group given 10.0 nmol BW245C (Table 1). Core body temperature was unchanged during occlusion and early reperfusion within each group.

BW245C does not significantly alter core body temperature in mice

Because PGD_2 has been implicated in temperature regulation, we wanted to determine whether it affected the temperature of the mice in our experiments. We injected DP1 agonist BW245C (10 nmol) ICV in a cohort of mice that did not undergo MCAO and monitored body temperature for 96 h. Core body temperature did not significantly differ between BW245C-treated mice and vehicle-treated mice at any time during the 96-h period (Fig. 4).

Augmentation of NMDA-induced brain damage in young $DP1^{-/-}$ is sustained in aged $DP1^{-/-}$ mice

Similar to our observations in young (8–10 weeks old) mice, aged (14–18 months old) $DP1^{-/-}$ mice were also more vulnerable than aged WT mice to NMDA-induced excitotoxicity, as noted by the greater

Fig. 2 Pretreatment with BW245C mitigates NMDA-induced brain damage. Mice were given a single ICV injection of vehicle ($n=6$) or 10 nmol ($n=9$), 25 nmol ($n=7$), or 50 nmol ($n=6$) BW245C 20 min before an injection of 15 nmol NMDA. Forty-eight hours later, mice were euthanized and brain sections were stained with Cresyl Violet to estimate the lesion volume. **a** Representative photographs of brain coronal sections from mice

pretreated with vehicle (*left panel*) or 10 nmol BW245C (*right panel*). **b** Analysis of the brain sections reveals a significant reduction in the mean lesion volume of BW245C-treated mice as compared with that of the vehicle-treated control group. Vehicle injection had no effect on NMDA-induced toxicity. * $P<0.05$, when compared with the vehicle-treated control group

lesion size in the knockout mice (Fig. 5a). Analysis of the sections revealed that NMDA-treated aged $DP1^{-/-}$ mice had a significantly ($P<0.05$) larger mean lesion volume of 4.98 mm^3 than did the WT mice, which had a mean lesion size of 3.24 mm^3 (Fig. 5b).

Discussion

Prostaglandin D_2 , the most abundant prostanoid of mammalian brain, has been shown to have roles in a variety of functions, from regulation of sleep and temperature to inflammatory disorders. However, its activity in neurodegeneration and neuroprotection has not been fully investigated. Our study was designed to evaluate the role of the PGD_2 $DP1$ receptor in cerebral ischemia and excitotoxicity. We found that genetic deletion of $DP1$ made mice more vulnerable to NMDA-induced toxicity. Interestingly, pharmacologi-

cal activation of $DP1$ by BW245C attenuated the lesion volume induced by NMDA. After establishing an effective dose curve, we wanted to know if the minimum effective dose of 10 nmol could lead to neuroprotection in MCAO. Indeed, 10 nmol of BW245C significantly reduced the size of the infarction following transient ischemia without affecting the monitored physiologic variables. Moreover, given the contribution of aging in stroke, we determined whether the neurotoxic effect of $DP1$ deletion was sustained in older $DP1^{-/-}$ mice. Our data suggest that, indeed, augmented NMDA-induced brain damage is persistent in aged $DP1^{-/-}$ mice. To our knowledge, this is the first in vivo study to show that NMDA-induced brain damage is augmented in $DP1^{-/-}$ mice in both young and aged animals and that $DP1$ receptor stimulation attenuates ischemic and excitotoxic brain damage.

We have previously shown that genetic deletion of the $DP1$ receptor leads to aggravated brain infarction

Fig. 3 Pretreatment with BW245C attenuates MCAO-induced brain infarction. Mice ($n=8$ per group) were injected with either vehicle or 10.0 nmol BW245C in the lateral ventricle before being subjected to 90-min MCAO and 4 days of reperfusion. **a** Representative photographs of TTC-stained brain slices of mice treated with vehicle (*left panel*) or 10 nmol BW245C (*right*

panel) show smaller infarct size in the BW245C-treated slice. **b** Analysis of the brain slices reveals that the hemispheric infarct volume was significantly attenuated in mice treated with 10 nmol BW245C compared with vehicle-treated mice. $*P<0.05$ compared with the vehicle-treated group

Table 1 Effect of BW245C on physiologic parameters

Parameter	Vehicle	10.0 nmol BW245C
Preischemia		
MABP	79±2	77±2
pH	7.23±0.03	7.25±0.03
PaCO ₂	43.7±1.5	49.2±2.5
PaO ₂	140±4	144±7
Ischemia		
MABP	74±2	77±2
pH	7.24±0.02	7.29±0.03
PaCO ₂	44.0±1.0	47.8±1.2
PaO ₂	135±4	143±7
Postischemia		
MABP	75±1	76±2
pH	7.27±0.02	7.23±0.03
PaCO ₂	45.2±1.2	46.8±2.4
PaO ₂	142±4	139±6

after MCAO (Saleem et al. 2007). Because excitotoxicity contributes substantially to the propagation of stroke damage, we went further in this study to determine the effect of DP1 deletion on NMDA-induced acute excitotoxicity. Figure 1 illustrates that the DP1 receptor is capable of minimizing NMDA-induced brain damage. This is the first study to determine the role of DP1 receptor activation by the agonist BW245C in acute excitotoxicity and MCAO paradigm in vivo. One other group has essentially shown that the DP1 agonist BW245C provides protection in rat E18 hippocampal cultures and in rat hippocampal slices (Liang et al. 2005). While the affinity of BW245C has been well characterized for mice and humans but not in rats, our in vivo studies using both DP1^{-/-} mice and the DP1 selective agonist in WT mice suggest that pharmacologic DP1 stimulation by BW245C is neuroprotective against excitotoxicity and stroke.

Fig. 4 Pretreatment with BW245C does not affect mouse core body temperature. BW245C (10 nmol) was injected ICV, and the body temperatures of the mice ($n=6$ per group) were recorded with an intra-abdominal radiofrequency probe every 10 min during the first 120 min after injection and then once

daily for 4 days while the mice were housed at room temperature. No significant differences in core body temperature were observed between the saline-treated and BW245C-treated groups

Fig. 5 Aggravated NMDA-induced toxicity is sustained in aged $DP1^{-/-}$ mice. One 15-nmol dose of NMDA was injected stereotactically into the striatum of 14–18-month-old WT and $DP1^{-/-}$ mice ($n=8$ per group). **a** Representative photographs of coronal sections of the brains of aged WT (left panel) and $DP1^{-/-}$ (right panel) mice show aggravated lesion in the latter. **b** Analysis of the Cresyl-Violet-stained brain sections shows that the NMDA-induced lesion size was significantly larger in the aged $DP1^{-/-}$ mice than in the aged WT mice. $*P<0.05$ when compared with the WT group

Furthermore, based on the susceptibility of the aged population to stroke, we hypothesized that the aggravated brain damage in young $DP1^{-/-}$ would be sustained in older $DP1^{-/-}$ mice, and our data very well supported this hypothesis. However, it should be noted that older WT mice had smaller lesion volumes than did young WT mice, and the same was true for $DP1^{-/-}$ mice. This finding can be explained by the fact that, with age, the population of glutamatergic receptors, such as the NMDA receptor, as well as glutamate content, decreases in corticostriatal and hippocampal areas (Banay-Schwartz et al. 1989; Saransaari and Oja 1995). Other studies also have shown that older animals have decreased NMDA receptor sensitivity in normal and excitotoxic conditions (Peterson and Cotman 1989; Wenk et al. 1991; Cohen and Muller 1992; Magnusson and Cotman 1993; see also reviews by Segovia et al. 2001; Dickstein et al. 2007). Our study supports these previous reports suggesting that age affects ischemic and excitotoxic brain damage outcomes. In our study, the decrease in excitotoxic injury in aged mice could have various causes, including a decrease in glutamatergic receptors, a decrease in glutamate content, and loss of dendritic spines. Thus, it is the first report that the neuroprotective role of the DP1 receptor is maintained in aged mice.

Few studies have shown a potential role for PGD_2 in regulation of vasodilatation. PGD_2 was shown to induce long-duration vasodilatation of the ascaris-sensitized pig airways without having major effects on the systemic arterial blood pressure (Alving et al. 1991); it was also shown to decrease relaxation of isolated rat mesenteric arteries (Shirahase et al. 2000). Activation of the DP1 receptor induced dilatation in contracted human pulmonary venous preparations (Walch et al. 1999) and enhanced nicotinic-acid-induced vasodilatation in mouse ear and nicotinic-acid-induced dermal flushing in humans (Cheng et al. 2006). Here, we did not observe any significant changes in the MABP or relative cerebral blood flow after ICV injection of BW245C in mice. We do not conclude that DP1 does not mediate vasodilatation; rather, we attribute our findings to the different testing paradigms and doses used in our study. Moreover, we did not observe any significant differences in other physiologic variables tested (Table 1).

PGD_2 also plays a role in radiation-induced hyperthermia temperature responses in rats (Kandasamy and

Hunt 1990). Microinjection of low-dose PGD_2 into the preoptic area or ICV injection caused hypothermia in rats but not in rabbits, whereas high-dose microinjection caused hyperthermia in rabbits but not in rats (Ito et al. 1989). However, we were unable to find a systematic study of the role of PGD_2 in temperature in mice. Considering the role that PGD_2 plays in the regulation of temperature, we monitored mouse core body temperature for 96 h after a 10 nmol BW245C injection. We did not observe any difference between the vehicle-treated and the drug-treated groups. Lower doses of BW245C also failed to produce significant temperature differences (data not shown). Although we did not investigate the role of DP1 in temperature regulation extensively, our data suggest that DP1 activation does not affect core body temperature in our experimental paradigm. It is noteworthy that a rat study predicted that despite its abundance in brain (Siren 1982), PGD_2 might not have an important role to play in central cardiovascular and thermal regulation. Moreover, Brus et al. (1980) reported no changes in behavior, body temperature, or blood pressure of Wistar rats after ICV injection of PGD_2 .

Various studies related to the protective role of prostaglandin E_2 receptors in paradigms of neuronal toxicity or animal models of neurodegeneration have indicated a potential role for increasing intracellular levels of cAMP and its downstream MAP kinase or protein kinase signaling pathways (McCullough et al. 2004; Echeverria et al. 2005; Ahmad et al. 2006c). In parallel with these reports, Liang et al. (2005) showed that DP1 receptor agonist BW245C protects against glutamate toxicity in cultured hippocampal neurons and organotypic slices in a cAMP-dependent fashion; they also showed that neurotoxic effects produced by glutamate in dispersed hippocampal neurons were abolished by protein kinase inhibitors. It is generally accepted that an increase in the level of intracellular cAMP leads to the activation of PKA, which subsequently leads to neuroprotection (Mattson et al. 1988; Hartikka et al. 1992; Sklair-Tavron and Segal 1993). Therefore, we believe that the protection observed in our studies could be partly mediated by an increase in intracellular cAMP concentration and activation of PKA signaling. As a proof of concept, this study involving in vivo experiments shows for the first time that DP1 activation is neuroprotective, and it establishes the first step toward defining the neuroprotective effects of DP1. However, more effort

is needed to fully elucidate the detailed neuroprotective mechanism of action of this receptor.

In conclusion, we have demonstrated that the DP1 receptor is vital to preventing brain damage. Both young and aged DP1^{-/-} mice were more vulnerable than controls to acute excitotoxicity, and activation of the DP1 receptor attenuated both acute and chronic brain damage in mice. The results provide evidence that the DP1 receptor is beneficial against stroke and excitotoxicity. Accordingly, selective targeting of the DP1 receptor could be considered as a potential therapeutic tool against stroke and excitotoxic brain damage.

Acknowledgements This work was supported in part by grants from the National Institutes of Health NS046400 and AG022971 (SD) and the American Heart Association 0830172N (ASA). We thank Claire Levine for assistance in the preparation of the manuscript and all members of the Doré lab team for assistance in this project.

Conflict of interest None of the authors have any conflict of interest associated with this work.

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