Behavioral/Systems/Cognitive

# Prostaglandin $E_2$ Acts on $EP_1$ Receptor and Amplifies Both Dopamine $D_1$ and $D_2$ Receptor Signaling in the Striatum

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**Dopamine is involved in multiple neural functions including motor control, reward and motivational processing, learning and reinforcement, and cognitive attention. Dopamine binds to two distinct classes of receptors, namely $D_1$ and $D_2$, to exert these functions. Various endogenous substances regulate dopamine signaling, although their physiological functions are not fully understood. Here, we examined the role of prostaglandin $E_2$ ($PGE_2$) and one of its receptors, $EP_1$, in dopaminergic function in the striatum. $EP_1$ was expressed in both preprodynorphin-containing $D_1$ and preproenkephalin-containing $D_2$ neurons, and $PGE_2$ was produced in striatal slices in response to both $D_1$ and $D_2$ dopamine receptor stimulation. $EP_1$-deficient mice exhibited significant suppression of hyperlocomotion induced by cocaine or SKF81297 (6-chloro-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine hydrobromide), a $D_1$ agonist, and significant attenuation of catalepsy induced by raclopride, a $D_2$ antagonist. Despite these behavioral defects, the extracellular concentration of dopamine was not suppressed in the striatum of $EP_1$-deficient mice, and the densities of $D_1$ and $D_2$ receptors in the striatum were not different between the two genotypes. Stimulation of the $D_1$ receptor induced phosphorylation of dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) at Thr34 in striatal slices, and the addition of indomethacin, a PG synthesis inhibitor, attenuated the $D_1$ agonist-induced increase in DARPP-32–Thr34 phosphorylation. The further addition of an $EP_1$ agonist restored the indomethacin-attenuated phosphorylation. Furthermore, both $D_1$- and $D_2$-mediated changes in the DARPP-32–Thr34 phosphorylation were attenuated in $EP_1^{-/-}$ slices. These results suggest that $PGE_2$ is formed in response to dopamine receptor stimulation in the striatum and amplifies both $D_1$ and $D_2$ receptor signaling via $EP_1$.**

*Key words:* **$EP_1$; DARPP-32; $D_1$; $D_2$; dopamine; prostaglandin; striatum**

## Introduction

Dopamine is implicated in various CNS functions including motor control, emotional processing, learning and reinforcement, and cognitive attention (Graybiel et al., 1994; Holland and Gallagher, 1999; Di Chiara, 2002; Wise, 2004; Everitt and Robbins, 2005). Dopaminergic neurons are located in several midbrain structures in mammals, such as substantia nigra pars compacta and ventral tegmental area, and innervate the striatum, the prefrontal cortex, and limbic structures such as the amygdala (Dahlström and Fuxe, 1964; Fallon and Moore, 1978). Dopamine exerts its functions by binding to two distinct classes of G-protein-coupled receptors (GPCRs), namely the $D_1$ receptors comprising the $D_1$ and $D_5$ subtypes and the $D_2$ receptors comprising the $D_2$, $D_3$, and $D_4$ subtypes (Missale et al., 1998). $D_1$ and $D_2$ receptors mediate specific functions through distinct intracellular signaling cascades (Missale et al., 1998; Nicola et al., 2000; Greengard, 2001; Nestler, 2005) and different patterns of expression (Goldman-Rakic, 1996; Gerfen, 2004). Furthermore, various neural pathways such as those of glutamate, serotonin, and acetylcholine as well as humoral factors such as adenosine interact with dopaminergic pathways and critically modulate $D_1$ and $D_2$ receptor signaling (Svenningsson et al., 2004). Elucidating neural and humoral networks surrounding dopaminergic neurons and regulation is therefore important for understanding functions of the dopaminergic nervous system as well as neurological and psychiatric disorders caused by its dysfunction.

We have been studying the physiological roles of prostaglandins (PGs). PGs including $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, $PGI_2$, and thromboxane $A_2$ ($TXA_2$) are a family of lipid mediators that are widely formed throughout our body and elicit versatile functions in a variety of processes including vascular homeostasis, inflammation, and reproduction (Narumiya et al., 1999). PGs bind to eight types and subtypes of GPCRs to exert their actions; they are the PGD receptor (DP), the four subtypes of PGE receptor ($EP_1$, $EP_2$, $EP_3$, and $EP_4$), the PGF receptor (FP), the PGI receptor (IP), and

Received July 18, 2007; revised Sept. 21, 2007; accepted Oct. 4, 2007.

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a grant from the National Institute of Biomedical Innovation of Japan, and a grant from ONO Research Foundation. We thank Prof. Takeshi Kaneko (Faculty of Medicine, Kyoto University, Kyoto, Japan) for antibodies to preproenkephalin, Ono Pharmaceutical Company (Osaka, Japan) for ONO-DI-004, Drs. Zhi-Li Huang and Yoshihiro Urade (Osaka Bioscience Institute, Osaka, Japan) for advice on microdialysis experiments, and Drs. Eri Segi and Yasuhiro Tanaka for discussion and comments. We also thank Teruhisa Fujiwara for animal care and breeding and Kimiko Nonomura, Yurie Kitagawa, and Tae Arai for assistance.

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DOI:10.1523/JNEUROSCI.3257-07.2007

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the TXA receptor (TP). Because of their specific localization and intracellular signaling pathways, each receptor can mediate a unique profile of actions in the body (Narumiya et al., 1999). The function of PGs in CNS has thus far been studied mostly in relation to sickness behaviors (Elmquist et al., 1997; Turnbull and Rivier, 1999). Our previous studies on each EP subtype-deficient mice individually revealed that $EP_3$ mediates febrile responses, and that both $EP_1$ and $EP_3$ are required for the neuroendocrine stress response (Ushikubi et al., 1998; Matsuoka et al., 2003). $EP_1$-deficient mice further exhibit enhanced aggression and impaired cliff avoidance, indicating that the $PGE_2$–$EP_1$ signaling controls impulsive behaviors under environmental and social stress (Matsuoka et al. 2005). Although these mice show enhanced dopamine turnover in the striatum and frontal cortex and administration of dopamine receptor antagonists suppresses their impulsivity, they do not show hyperlocomotion. Here, we have analyzed the role of $EP_1$ in the dopaminergic signaling in the striatum and related behaviors. Our data suggest that $PGE_2$ is formed in response to dopamine receptor stimulation in the striatum and regulates signaling and function of both $D_1$ and $D_2$ receptors via $EP_1$.

## Materials and Methods

*Mice.* Mice lacking the *Ptger1* gene encoding $EP_1$ ($EP_1^{-/-}$) were generated and backcrossed >10 generations to the C57BL/6CrSlc background as described previously (Matsuoka et al., 2005). Male littermates 2–3 months of age having $EP_1^{-/-}$ and wild-type genotypes derived from the same $EP_1^{+/-}$ parents were used for each comparison group in all the experiments, except those in Figure 6 and supplemental Figure 2 (available at www.jneurosci.org as supplemental material), in which age-matched, male C57BL/6CrSlc mice (SLC, Shizuoka, Japan) were used as the wild-type control. All animal care and use was in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine or that of Kurume University Graduate School of Medicine.

In situ *hybridization and immunohistochemistry.* *In situ* hybridization was performed using the standard method with an $^{35}S$-labeled antisense riboprobe matching the entire length of mouse $EP_1$ cDNA as described previously (Furuyashiki et al., 1999). Control slides were incubated with the radioactive probe in the presence of a 200 times excess amount of nonradioactive riboprobe from the same template. Immunohistochemistry was performed as described previously (Lee et al., 1997) with modifications. Under deep anesthesia with sodium pentobartiturate injected intraperitoneally (60 mg/kg; Dainippon Pharmaceutical, Osaka, Japan), mice were perfused transcardially with 0.9% sodium chloride in 5 mM phosphate buffer (PBS; pH 7.4), followed by 4% formaldehyde in 0.1 M sodium phosphate, pH 7.4. After incubation in 30% sucrose in PBS for cryoprotection, brains were cut at coronal planes at 20 μm thickness on a microtome frozen at −20°C. The brain sections were incubated for 36 h at 4°C with a rabbit polyclonal antibody to $EP_1$ (1:250; 101740; Cayman Chemical, Ann Arbor, MI), followed by incubation with HRP-conjugated goat anti-rabbit IgG (1:200 dilution; 111-035-144; Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. The incubation with antibodies was performed in PBS containing 0.25% Triton X-100, 0.25% λ-carrageenan, 10% goat serum, and 1% BSA, and followed by a rinse with PBS containing 0.1% Triton X-100. For double staining, the sections were further incubated overnight at 4°C with a guinea pig polyclonal antibody to preprodynorphin (PPD) (1:200 dilution; AB5519, Chemicon, Temecula, CA) or that to preproenkephalin (PPE) (1:200 dilution; a gift from Dr. Takeshi Kaneko, Kyoto University, Kyoto, Japan) (Lee et al., 1997). Signals for $EP_1$ and PPD/PPE were visualized with fluorescein-conjugated Tyramide (PerkinElmer, Boston, MA) and Cy3-conjugated anti-guinea pig IgG antibody (1:500 dilution; 706-165-148; Jackson ImmunoResearch), respectively. Fluorescence images were acquired on a LSM510 laser-scanning confocal microscope (Zeiss, Oberkochen, Germany). The specificity of the $EP_1$ signal was examined by blocking with an epitope peptide as well as on tissues from $EP_1$-deficient mice (see Results).

*Perfusion of striatal slices and quantification of $PGE_2$.* Striatal slices of 300 μm thickness were prepared from wild-type mice as described previously (Nishi et al., 1997). The slices were loaded onto the chambers and perfused with Krebs-$HCO_3^-$ buffer (124 mM NaCl, 4 mM KCl, 26 mM $NaHCO_3$, 1.5 mM $CaCl_2$, 1.25 mM $KH_2PO_4$, 1.5 mM $MgSO_4$, 10 mM D-glucose, pH 7.4) saturated with 95% $O_2$ and 5% $CO_2$ at a flow rate of 0.5 ml/min. Some animals and their slices were treated with indomethacin, a cyclooxygenase (COX) inhibitor that blocks the production of PGs. For the indomethacin-treated controls, slices were prepared from wild-type mice administered with 5 mg/kg indomethacin (Nacalai Tesque, Kyoto, Japan) intraperitoneally 30 min before decapitation, and were perfused with Krebs-$HCO_3^-$ buffer containing 1 mM indomethacin. After equilibration for 60 min, the perfusing buffer was changed to Krebs-$HCO_3^-$ buffer containing either vehicle or indicated concentrations of 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzazepine hydrobromide (SKF81297) (Sigma-Aldrich, St. Louis, MO), or quinpirole (Sigma-Aldrich), or both. After the slices were exposed to the drug-containing buffer, perfusates were collected for 5 min in chilled tubes containing 600 μl of ethanol and 700 μl of 0.1N hydrochloric acid. [5,6,8,11,12,14,15(*n*)-$^3$H]$PGE_2$ (7.4 TBq/mmol; Amersham Biosciences, Piscataway, NJ) (10,000 cpm) was then added to the samples as a tracer for monitoring recovery, and the samples were centrifuged at 3000 × *g* for 10 min at 4°C. The supernatants were applied to preconditioned Sep-Pak plus cartridges (WAT0200515; Waters, Milford, MA), which were washed serially with 15% ethanol, petroleum ether, and, finally, 5 ml of methyl formate as described previously (Powell, 1980). The methyl formate fraction was recovered in a glass vial and stored at −80°C until use. Methyl formate was then evaporated, and the residues were suspended in 150 μl of water/methyl formate (80:20, v/v). Samples were then subjected to HPLC on a Cosmosil $5C_{18}$ column (4.6 × 150 mm; Nacalai Tesque) with water/acetonitrile/acetic acid (67:33:0.01, v/v/v). Fractions corresponding to the elution position of authentic $PGE_2$ were collected, and, after evaporation, residues were suspended in 200 μl volume of an assay buffer (0.1 M phosphate buffer, pH 7.5, containing 0.9% bovine serum albumin and 0.5% kathon). The $PGE_2$-like immnoreactivity was then determined using an enzyme immunoassay (EIA) kit (GE Healthcare, Piscataway, NJ).

*Behavioral analysis.* Acute and chronic psychostimulant effects of cocaine were tested as described previously (Hikida et al., 2001). For acclimation to the injection procedure, mice were injected intraperitoneally with saline and placed in a transparent, plastic test chamber (22 × 32 × 13.5 $cm^3$) for 10 min once a day on days 1 and 2. From day 3 to day 7, mice were administered cocaine (10 or 20 mg/kg; Takeda Pharmaceutical, Osaka, Japan) or vehicle (0.9% sodium chloride) and placed in the test chamber for 10 min once a day. For SKF81297-induced hyperlocomotion (Xu et al., 1994), mice were habituated to a transparent, plastic test chamber (46 × 46 × 30.3 $cm^3$) for 1 h. They then received intraperitoneal injection of SKF81297 (2.5 or 5 mg/kg) or vehicle (0.9% sodium chloride) and were immediately returned to the test chamber for an additional 1 h observation period. Locomotor activity was measured based on interruption of infrared beams (Melquest, Toyama, Japan). Raclopride-induced catalepsy was measured as described previously (Pertwee, 1972). Mice were injected intraperitoneally with raclopride (0.25, 0.5, 1, and 2 mg/kg; Sigma-Aldrich) or vehicle (0.9% sodium chloride). Behavior was video-recorded for 5 min from 30 min after drug injection, and the duration of catalepsy was determined *post hoc*. Catalepsy was defined as the total motionless time on the wire ring. All behavioral tests were performed and analyzed by experimenters blinded to the mouse genotype. A total volume of intraperitoneal injection was set at 8 ml/kg by adjusting drug concentrations and matched across individuals in the same comparison.

*Whole-animal studies.* Mice were injected intraperitoneally with vehicle (0.9% sodium chloride), cocaine (10 mg/kg), or SKF81297 (2.5 mg/kg) at 15 min before decapitation. After decapitation, the heads of mice were immediately immersed in liquid nitrogen for 30 s. Rostral parts of the frozen heads were cut to the level of the striatum on dry ice, and striatal tissues were punched out and stored at −80°C

until use. For quantification of $PGE_2$, frozen striata were weighed, and extracted by homogenization of the tissues in ethanol containing 0.1 M hydrochloric acid. The extracts were then mixed with a tracer of [$^3$H]$PGE_2$ and subjected to quantification. Alternatively, frozen striata were used for analysis of dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) phosphorylation as described below.

*Analysis of DARPP-32 phosphorylation.* Experiments were performed as described previously (Nishi et al., 1997). After decapitation, the brain was rapidly removed, and coronal striatal slices were prepared at 350 $\mu$m thickness using a vibrating blade microtome, VT1000S (Leica Microsystems, Nussloch, Germany). The slices were preincubated at 30°C for 60 min in Krebs-$HCO_3^-$ buffer saturated with 95% $O_2$ and 5% $CO_2$, and were incubated with drugs as specified in each experiment. ONO-DI-004 was obtained from Ono Pharmaceutical (Osaka, Japan). To terminate the incubation, slices were frozen on dry ice, and stored at −80°C until use. For immunoblot, the tissues were sonicated in boiling 1% SDS, and boiled for 10 min. Each sample of 100 $\mu$g protein was subjected to SDS-PAGE (10% polyacrylamide gels), and separated proteins were transferred to nitrocellulose membranes (Nishi et al., 1997). The membranes were incubated with mAb-23, a monoclonal antibody to Thr34-phosphorylated DARPP-32 (1:750 dilution) (Snyder et al., 1992). The membrane was then incubated with a goat anti-mouse Alexa 680-linked IgG (1:5000; Invitrogen, Eugene, OR) or a goat anti-mouse IRDye 800-linked IgG (1:5000; Rockland, Gilbertsville, PA). Antibody binding was quantified with the use of an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). For each experiment, values were normalized to those of control slices without $D_1$ or $D_2$ stimulation. To determine the total amount of DARPP-32 in samples, C24-5a monoclonal antibody to DARPP-32 (1:22,500 dilution) (Hemmings et al., 1984), which is not phosphorylation state-specific, was used for reblotting the membrane. None of the experimental manipulations in the present study altered the total amount of DARPP-32.

*Statistical analysis.* All data are shown as means ± SEM. Comparison of two groups was analyzed using unpaired two-tailed Student's *t* test. One-way or two-way ANOVA was performed for each comparison followed by Tukey's or Bonferroni's *post hoc* tests for evaluation of pairwise group differences. A value of $p < 0.05$ was considered statistically significant. The analyses were performed by Prism 4.0 software (GraphPad, San Diego, CA).

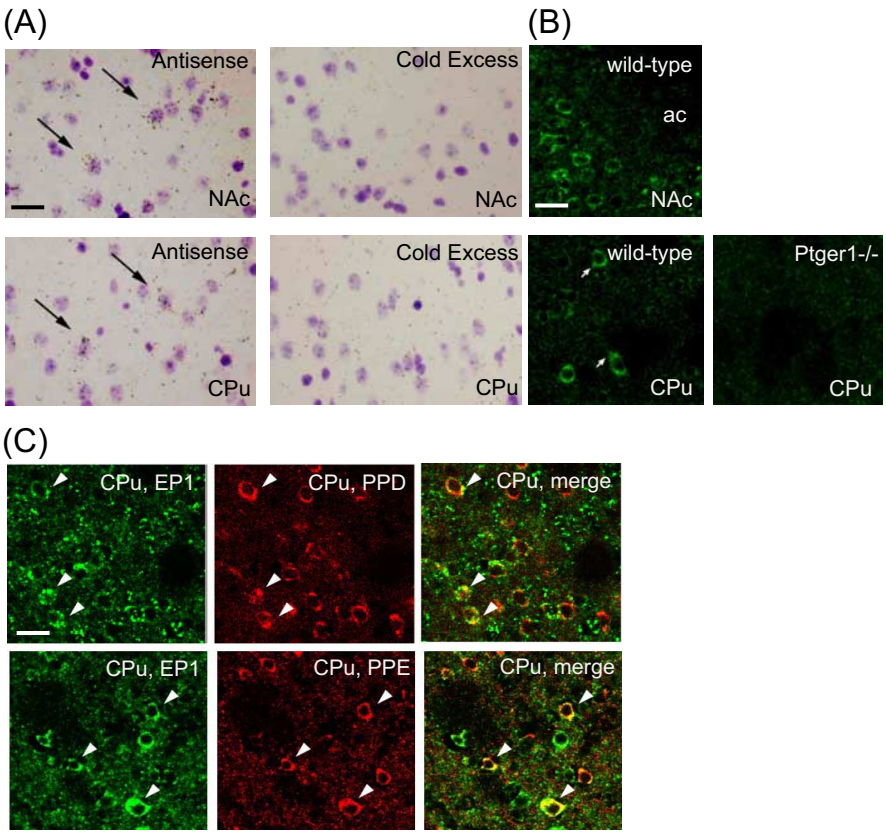


**Figure 1.** Localization of $EP_1$ in the striatum. ***A***, *In situ* hybridization in the dorsal (CPu) and ventral (NAc) striatum. Signals with the $EP_1$ antisense riboprobes are indicated by arrows. No significant signals were detected in an excess of nonradioactive riboprobes from the same template (Cold Excess). ***B***, Immunofluorescence. Signals for $EP_1$ detected in the soma and proximal processes (indicated by arrows) of cells in the dorsal (CPu) and ventral (NAc) striatum of wild-type mice are reduced to the background level in the striatum from $EP_1$-deficient mice. ac, Anterior commissure. ***C***, Double staining for $EP_1$ and PPD (top panels) or $EP_1$ and PPE (bottom panels). Signals for $EP_1$ and PPD or PPE are shown in green (left panels) and red (middle panels), respectively, and the merged images are shown in right. The arrowheads indicate neurons showing colocalization of the two signals. Scale bars, 20 $\mu$m.

## Results

### $EP_1$ is expressed in both $D_1$- and $D_2$-containing neurons in the striatum

We first examined expression and localization of $EP_1$ in the striatum. *In situ* hybridization using radioactive $EP_1$ antisense riboprobe exhibited silver grains diffusely distributed over cell bodies of a large number of cells in both ventral and dorsal striatum, namely the nucleus accumbens (NAc) and the caudate putamen (CPu). The size and shape of these cells indicate that they are striatal neurons. Most of the grains over these cells were abolished by the addition of an excess amount of cold riboprobes (Fig. 1*A*), indicating expression of $EP_1$ in a majority of neurons in the striatum. We then confirmed this finding by immunostaining. Because the $EP_1$ immunoreactivity was not of a high amount, we amplified the antigen–antibody complex by the fluorescein-conjugated Tyramide-HRP system. The amplified signals exhibited both diffuse and punctate accumulation in the cytoplasm and proximal processes of a majority of neuron-like cells in the striatum (Fig. 1*B*). In addition, small dot-like signals forming ill defined networks were found over the tissue. Most of the former signals and not the latter were absent in the striatum of $EP_1$-deficient mice, suggesting the former represent $EP_1$ receptor immunoreactivity and the latter background. These results suggested that $EP_1$ is expressed and present in a number of neurons in the striatum. Over 90% of striatal neurons in rodents are GABAergic projection neurons called medium spiny neurons (MSNs) (Gerfen, 2004). MSNs are composed of at least two neurochemically distinct populations: those expressing $D_1$ receptors and PPD and others expressing $D_2$ receptors and PPE (Gerfen and Young, 1988; Lee et al., 1997). We therefore conducted double immunohistochemistry for $EP_1$ and these marker proteins. Double staining for $EP_1$ and PPD or $EP_1$ and PPE showed that signals for $EP_1$ colocalized with those for PPD in some neurons and with those for PPE in other neurons, suggesting that $EP_1$ is expressed in both PPD-containing $D_1$ and PPE-containing $D_2$ MSNs (Fig. 1*C*). Quantitative analysis revealed that, in the CPu, $EP_1$ was expressed in 61% of PPD-positive neurons and 55% of PPE-positive neurons and that, in the NAc, $EP_1$ was expressed in 71% of PPD-positive neurons and 79% of PPE-positive neurons.

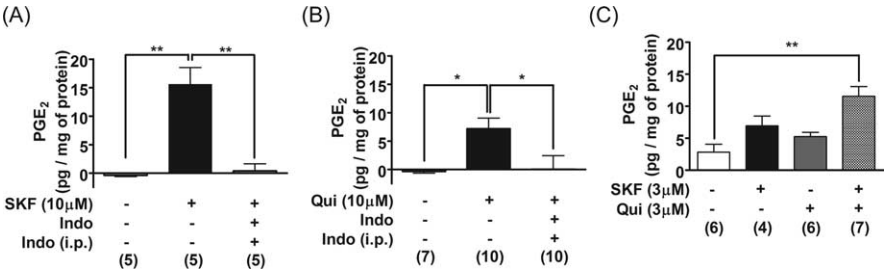


**Figure 2.** Prostaglandin $E_2$ is produced in striatal slices in response to dopamine receptor stimulation. Striatal slices were prepared from wild-type mice, perfused with Krebs-$HCO_3^-$, and stimulated with either vehicle (control), a $D_1$ agonist, SKF81297 (10 $\mu$M) (SKF) (***A***), or a $D_2$ agonist, quinpirole (10 $\mu$M) (Qui) (***B***), or both (3 $\mu$M each) (***C***) for 5 min. The amount of $PGE_2$ in the perfusates was measured by HPLC-linked enzyme immunoassay kit as described in Materials and Methods. As alternative controls, striatal slices were prepared from indomethacin-injected mice, pretreated with 1 mM indomethacin (Indo) for 60 min, and stimulated with SKF81297 or quinpirole in the continued presence of indomethacin. The numbers of mice used in experiments are shown below each column. Error bars indicate SEM. $^*p < 0.05$; $^{**}p < 0.01$.

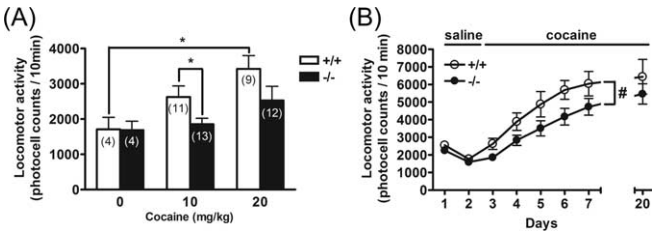


**Figure 3.** ***A***, Suppression of cocaine-induced hyperlocomotion in $EP_1$-deficient mice. Wild-type (+/+) and $EP_1$-deficient (−/−) mice were injected with either vehicle or 10 or 20 mg/kg cocaine, and locomotor activity was measured over 10 min. The numbers of animals are shown in each column. $^*p < 0.05$. ***B***, Reduced hyperlocomotion in $EP_1$-deficient mice during and after sensitization with repeated cocaine injections. Wild-type (+/+; $n = 11$) and $EP_1$-deficient mice (−/−; $n = 13$) were administered vehicle once a day on days 1 and 2, and then 10 mg/kg cocaine from day 3 to day 7. After 2 weeks of cocaine-free period, the mice were challenged again with 10 mg/kg cocaine on day 20. The locomotor activity was measured over 10 min after each injection. The data at 10 mg/kg in ***A*** were adopted from those at day 3 in ***B***. A difference between the genotypes was maintained over the entire testing periods ($F_{(1,4)} = 4.925$; $^{\#}p = 0.037$). Error bars indicate SEM.

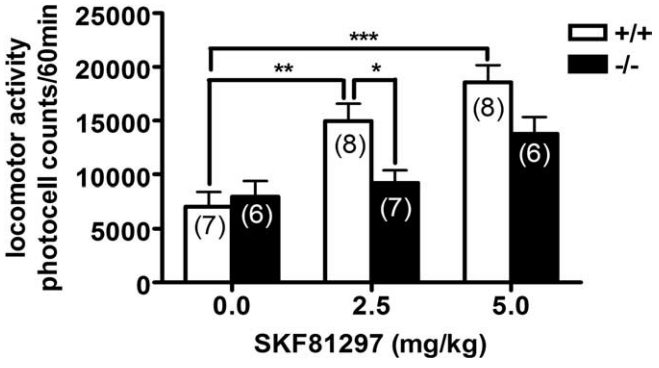


**Figure 4.** Suppression of hyperlocomotion induced by $D_1$ receptor stimulation in $EP_1$-deficient mice. After a 1 h habituation, wild-type (+/+) and $EP_1$-deficient (−/−) mice were injected intraperitoneally with either saline or 2.5 or 5 mg/kg SKF81297, and the locomotor activity was measured for 60 min. The numbers of animals are shown in each column. Error bars indicate SEM. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ for indicated comparison.

### Prostaglandin $E_2$ is produced in striatal slices in response to dopamine receptor stimulation

The above findings indicate a possibility that $PGE_2$ is synthesized and acts on $EP_1$ in the striatum. To test this hypothesis, we prepared striatal slices from wild-type mice, incubated them with either vehicle, a $D_1$ agonist, SKF81297, or a $D_2$ agonist, quinpirole, and examined production of $PGE_2$. $PGE_2$ was extracted from the reaction medium by the use of a Sep-Pak plus cartridge and purified by reversed-phase HPLC, and the $PGE_2$-like immunoreactivity in the HPLC fraction was measured by EIA. Authenticity of the $PGE_2$-like immunoreactivity was further verified by its decrease in samples obtained from indomethacin-treated mice and incubated with indomethacin. The addition of either SKF81297 or quinpirole significantly increased $PGE_2$ production in the striatal slices, and this increase was abolished by treatment with indomethacin (Fig. 2*A*,*B*). Furthermore, when 3 $\mu$M each of SKF81297 and quinpirole were added together, the $PGE_2$ production under this condition was about the sum of those induced by each compound alone (Fig. 2*C*). These results suggest that $PGE_2$ is produced in the striatum in response to both $D_1$ and $D_2$ dopamine receptor stimulation and the effect of $D_1$ and $D_2$ receptor stimulation is additive.

### $EP_1$ deficiency alters behaviors mediated by dopaminergic signaling in the striatum

To elucidate the function of $EP_1$ in the striatum *in vivo*, we used acute cocaine injection. Acute administration of cocaine leads to hyperlocomotion, which is mediated primarily by high synaptic concentration of dopamine because of blockade of dopamine transporters in the striatum (Giros et al., 1996; Pierce and Kalivas, 1997). We used wild-type and $EP_1$-deficient littermates from heterozygous mating, and, after acclimation to injection procedures over 2 d, injected either saline or cocaine (10 or 20 mg/kg) intraperitoneally to these mice. We then tested their locomotor activity in the next 10 min. Cocaine induced hyperlocomotion in wild-type mice in a dose-dependent manner. $EP_1$-deficient mice also showed a dose-dependent response to cocaine injection, but hyperlocomotion in these mice was significantly reduced compared with that found in wild-type mice, whereas the locomotor activity on saline injection did not differ between the genotypes (Fig. 3*A*). We next examined whether $EP_1$ is also involved in behavioral sensitization induced by repeated cocaine exposure (Pierce and Kalivas, 1997). Cocaine (10 mg/kg) was injected intraperitoneally once a day into wild-type and $EP_1$-deficient littermates over 5 consecutive days from day 3 to day 7. Daily exposure to cocaine induced sensitization (i.e., a gradual increase in locomotor response to each-day injection of the same dose of cocaine in both wild-type and $EP_1$-deficient mice). Furthermore, after 2 week abstinence, both genotypes similarly showed retention of previous sensitization. However, the $EP_1$-deficient mice persistently showed significantly less locomotor activity in response to each day cocaine injection over the entire test period (Fig. 3*B*). These data suggest that $EP_1$ is not involved in sensitization to cocaine per se, but participates in elicitation of acute locomotor activity induced by each injection of cocaine. We next injected a $D_1$-selective agonist, SKF81297, and examined effects of the $EP_1$ deficiency on hyperlocomotion induced by $D_1$ receptor stimulation (Bordi and Meller, 1989). SKF81297 stimulated locomotion of wild-type mice in a dose-dependent manner, which lasted over 1 h after injection (Fig. 4). Compared with wild-type mice, $EP_1$-deficient mice showed reduced hyperlocomotion in response to

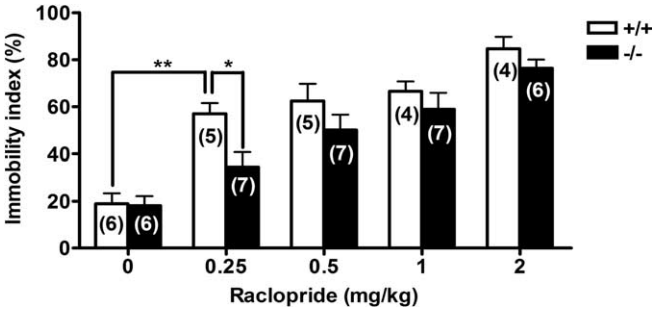


**Figure 5.** Suppression of catalepsy induced by $D_2$ receptor inhibition in $EP_1$-deficient mice. Wild-type (+/+) and $EP_1$-deficient (−/−) mice were injected intraperitoneally with either saline or 0.25, 0.5, 1.0, or 2.0 mg/kg raclopride, and immobility time was measured for 5 min from 30 min after the injection. The numbers of animals are shown in each column. Error bars indicate SEM. $*p < 0.05$; $**p < 0.01$.

SKF81297. The reduction in $EP_1$-deficeint mice was statistically significant at the dose of 2.5 mg/kg SKF81297, and became less apparent at the higher dose. These findings indicate that $PGE_2$ is formed *in vivo* in the striatum in response to $D_1$ receptor stimulation and modulates mouse behavior induced by this signaling. To confirm this hypothesis, we treated wild-type mice for 15 min with 2.5 mg/kg SKF81297. Mice were then decapitated, and production of $PGE_2$ was measured in the striatum punched out from frozen brain. We found that a considerable amount of $PGE_2$ was present already under basal conditions, but that administration of SKF81297 significantly increased $PGE_2$ production in this brain region (1.98 ± 0.10 and 2.38 ± 0.10 pg/mg of tissue for control and SKF81297-treated mice, respectively; $n = 4$ for each group; $p = 0.034$). A similar treatment with cocaine (10 mg/kg) tended to increase striatal $PGE_2$ production (2.08 ± 0.08 pg/mg of tissue; $n = 4$; $p = 0.485$), but the increase was not statistically significant compared with the vehicle-treated control group.

Given expression of $EP_1$ in not only $D_1$-containing MSNs but also $D_2$-containig MSNs, we next examined effects of $EP_1$ deficiency on a behavioral paradigm induced by a $D_2$-selective drug. To this end, we tested catalepsy induced by a $D_2$-selective antagonist, raclopride (Pertwee, 1972). The addition of raclopride dose-dependently increased immobility time (i.e., catalepsy) in wild-type mice. Although raclopride also dose-dependently increased catalepsy in $EP_1$-deficient mice, this response was significantly suppressed in the $EP_1$-deficient mice at a dose of 0.25 mg/kg (Fig. 5).

### Extracellular dopamine concentration and ligand binding properties of $D_1$ and $D_2$ receptors in the striatum of $EP_1$-deficient mice

Neurotransmitter signaling depends on both release of a neurotransmitter from the presynaptic terminal and its binding to the cognate receptor at the postsynaptic site. We analyzed whether $EP_1$ deficiency affects either of these processes. We first addressed the presynaptic mechanism by measuring the extracellular dopamine content in the ventral striatum of wild-type and $EP_1$-deficient mice by microdialysis. The striatum was perfused *in vivo* with artificial CSF through a microdialysis probe, and the content of dopamine recovered in the effluent was determined. The dopamine content in the dialysates from $EP_1$-deficient mice tended to be higher than that from wild-type mice, although there was no statistically significant difference (supplemental Fig. 1*A*, available at www.jneurosci.org as supplemental material). The dopamine contents from both genotypes of animals increased after acute cocaine injection (10 mg/kg), the condition in which a significantly reduced hyperlocomotion in $EP_1$-deficient mice was observed (Fig. 3). When normalized to the baseline level before cocaine injection, both groups exhibited a similar rise in the dopamine content (supplemental Fig. 1*B*, available at www.jneurosci.org as supplemental material). Although we did not determine absolute concentration of dopamine by equilibrium microdialysis, these results suggest that the $EP_1$ deficiency did not reduce the synaptic concentration of dopamine compared with that in wild-type mice before and after the cocaine injection.

We next examined ligand binding properties of $D_1$ and $D_2$ receptors. The membrane fraction was prepared from the striatum of wild-type and $EP_1$-deficient mice, and subjected to radioligand binding assay using [$^3$H]SCH23390 and [$^3$H]spiperone for $D_1$ and $D_2$ receptors, respectively (Glowinski and Iversen 1966; List and Seeman, 1981; Charifson et al., 1988) (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). The [$^3$H]SCH23390 binding exhibited a single class of binding; the $K_d$ and $B_{max}$ values were 0.421 ± 0.080 and 0.555 ± 0.063 nM ($p = 0.266$) and 1311 ± 125 and 1495 ± 221 fmol/mg protein ($p = 0.489$) for wild-type and $EP_1$-deficient mice, respectively. The [$^3$H]spiperone binding exhibited two classes of binding, high-affinity binding and low-affinity binding, which apparently represent $D_2$ and 5-$HT_2$, respectively. The $K_d$ and $B_{max}$ values of the former binding were 0.212 ± 0.057 and 0.286 ± 0.132 nM ($p = 0.634$) and 287 ± 48 and 347 ± 106 fmol/mg protein ($p = 0.633$) for wild-type and $EP_1$-deficient mice, respectively, and the $K_d$ and $B_{max}$ values of the latter binding are 1.37 ± 0.61 and 1.19 ± 0.48 pM ($p = 0.829$) and 420 ± 194 and 389 ± 243 fmol/mg protein ($p = 0.926$) for wild-type and $EP_1$-deficient mice, respectively.

### $EP_1$ augments both $D_1$- and $D_2$-mediated effects on DARPP-32 Thr34 phosphorylation in striatal slices

Because the above analysis could not reveal significant differences in dopamine release or binding properties of $D_1$ and $D_2$ receptors in the striatum between wild-type mice and $EP_1$-deficient mice, we next examined the downstream signaling of $D_1$ and $D_2$ receptors. To address this issue, we used striatal slices and examined DARPP-32 phosphorylation at Thr34, a critical downstream step in $D_1$ and $D_2$ receptor signaling (Svenningsson et al., 2004). Treatment of the slices with SKF81297 increased the level of phospho-Thr34 DARPP-32 (Fig. 6*A*), as previously shown (Nishi et al., 2000). We then examined the effect of indomethacin on the SKF81297-induced increase in this phosphorylation. Treatment of slices with indomethacin significantly attenuated the SKF81297-induced increase in DARPP-32 Thr34 phosphorylation (Fig. 6*A*), indicating the involvement of endogenously formed PGs in $D_1$ receptor signaling in the striatum. Consistently, when an $EP_1$-selective agonist, ONO-DI-004, was added to the indomethacin-treated slices, ONO-DI-004 restored the SKF81297-induced increase in DARPP-32 Thr34 phosphorylation in a concentration-dependent manner (Fig. 6*B*), whereas ONO-DI-004 alone did not affect DARPP-32 Thr34 phosphorylation (data not shown).

We next examined the effect of SKF81297 on DARPP-32 phosphorylation in $EP_1$-deficient mice. Treatment of striatal slices with SKF81297 for 2 min stimulated DARPP-32 Thr34 phosphorylation by approximately threefold in slices from both wild-type and $EP_1$-deficient mice. However, the subsequent increase in the Thr34 phosphorylation observed in slices from wild-type mice (by approximately sixfold at 5 min of incubation) was

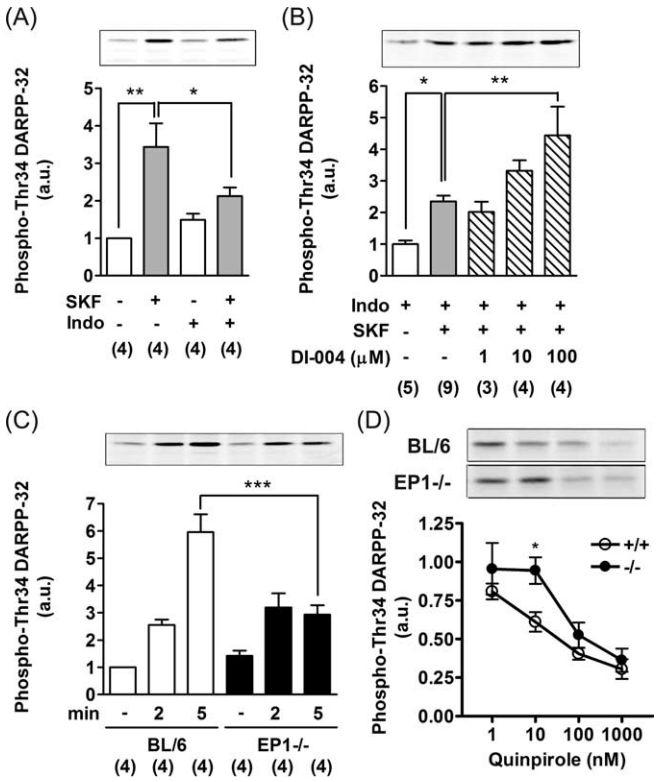


**Figure 6.** Effects of an $EP_1$-selective agonist and of $EP_1$ deficiency on dopamine $D_1$ and $D_2$ receptor-mediated changes in DARPP-32 Thr34 phosphorylation in striatal slices. ***A***, Effect of indomethacin on $D_1$ receptor-stimulated DARPP-32 Thr34 phosphorylation in striatal slices from wild-type mice. After incubation for 30 min with or without 100 $\mu$M indomethacin (Indo), slices were incubated for additional 5 min with 1 $\mu$M SKF81297 (SKF) or vehicle. Typical immunoblots for detection of phospho-Thr34 DARPP-32 are shown in top panels. The levels of phospho-Thr34 DARPP-32 were normalized to values obtained from untreated slices (a.u., arbitrary units). ***B***, Effects of an $EP_1$ agonist, ONO-DI-004, on $D_1$ receptor stimulated DARPP-32 Thr34 phosphorylation in PG-depleted slices. Striatal slices, prepared from indomethacin-injected mice, were incubated with 100 $\mu$M indomethacin for 60 min to block PG synthesis. In the continued presence of indomethacin, the slices were incubated with 0, 1, 10, or 100 $\mu$M ONO-DI-004 for 10 min, and SKF81297 (1 $\mu$M) was added at 5 min of incubation. ***C***, Effects of a $D_1$ receptor agonist on DARPP-32 Thr34 phosphorylation in striatal slices from wild-type and $EP_1$-deficient mice. Striatal slices, prepared from wild-type (BL/6) and $EP_1$-deficient ($EP_1^{-/-}$) mice, were incubated with 1 $\mu$M SKF81297 for 0, 2, or 5 min. The numbers of slices used in experiments ***A–C*** are shown below each column. ***D***, Effects of a $D_2$ receptor agonist, quinpirole, on DARPP-32 Thr34 phosphorylation in striatal slices from wild-type and $EP_1$-deficient mice. Striatal slices, prepared from wild-type (+/+) and $EP_1$-deficient (−/−) mice, were incubated with either vehicle or quinpirole (1, 10, 100, or 1000 nM) for 5 min. The data were normalized to values from vehicle-treated slices in each genotype. $n = 5–9$. Error bars indicate SEM. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

blunted in the slices from $EP_1$-deficient mice (Fig. 6*C*). These results suggest that SKF81297 stimulates $PGE_2$ production and that the $PGE_2$ produced acts on $EP_1$ to augment $D_1$ receptor signaling in striatal slices in a positive-feedback manner.

Activation of $D_2$ receptors is known to suppress DARPP-32 phosphorylation at Thr34 (Nishi et al., 1997). Consistent with previous reports, treatment of striatal slices with quinpirole decreased DARPP-32 Thr34 phosphorylation in a concentration-dependent manner (Fig. 6*D*). The ability of quinpirole to decrease DARPP-32 Thr34 phosphorylation was significantly attenuated at 10 nM in slices from $EP_1$-deficient mice compared with that in slices from wild-type mice, although the effects of quinpirole were similar at higher concentration. These results suggest that the $PGE_2$–$EP_1$ signaling augments $D_2$ receptor signaling, leading to the suppression of DARPP-32 phosphorylation at Thr34. Together, our data suggest that $EP_1$ interacts with and augments both $D_1$ and $D_2$ receptor signaling in striatal neurons.

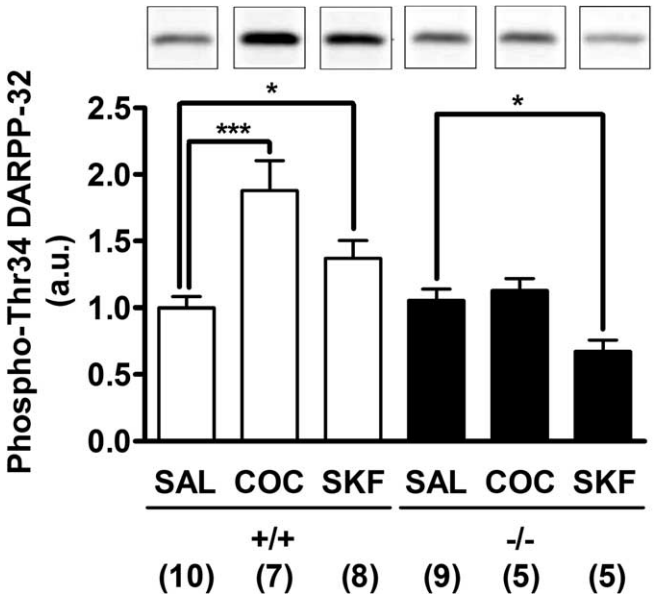


**Figure 7.** Attenuation of DARPP-32 Thr34 phosphorylation induced by cocaine or a $D_1$ receptor agonist in the striatum of $EP_1$-deficient mice *in vivo*. Wild-type (+/+) and $EP_1$-deficient (−/−) mice were injected (intraperitoneally) with vehicle (0.9% sodium chloride) (SAL), cocaine (10 mg/kg) (COC), or a $D_1$ receptor agonist, SKF81297 (2.5 mg/kg) (SKF). Fifteen minutes after drug injection, the mice were decapitated. The striatum was dissected from frozen brain, and the level of phospho-Thr34 DARPP-32 was analyzed. Typical immunoblots for detection of phospho-Thr34 DARPP-32 are shown in the top panels. The data were normalized to values from saline-treated samples in each genotype (a.u., arbitrary units). The numbers of mice used in experiments are shown below each column. Error bars indicate SEM. $^*p < 0.05$; $^{***}p < 0.001$.

### Attenuation of cocaine or $D_1$ agonist-induced DARPP-32 Thr34 phosphorylation in the striatum of $EP_1$-deficient mice *in vivo*

To investigate the role of $PGE_2$–$EP_1$ signaling in the regulation of DARPP-32 phosphorylation *in vivo*, wild-type and $EP_1$-deficient mice were treated for 15 min with either cocaine (10 mg/kg) or SKF81297 (2.5 mg/kg), conditions in which stimulation of locomotor activity was attenuated in $EP_1$-deficient mice (Figs. 3, 4). In wild-type mice, administration of both cocaine and SKF81297 significantly increased the level of DARPP-32 Thr34 phosphorylation in the striatum (Fig. 7). In contrast, in $EP_1$-deficient mice, administration of cocaine did not increase and administration of SKF81297 decreased DARPP-32 Thr34 phosphorylation. These results indicate that the $PGE_2$–$EP_1$ pathway plays an essential role in the regulation of dopaminergic signaling in the striatum *in vivo*.

## Discussion

In this study, we used $EP_1$-deficient mice and an $EP_1$-selective agonist, and examined whether the $PGE_2$–$EP_1$ pathway regulates dopaminergic signaling and function in the striatum. We found that $EP_1$ is expressed in more than one-half of MSNs in the striatum, that $PGE_2$ is produced in the striatum in response to stimulation with a $D_1$ or $D_2$ agonist, and that the $PGE_2$–$EP_1$ pathway facilitates both $D_1$ and $D_2$ signaling as assayed by DARPP-32 Thr34 phosphorylation *in vitro* in striatal slices. The *in vivo* relevance of these *in vitro* findings was underscored by significant attenuation of cocaine- or $D_1$ agonist-induced hyperlocomotion, $D_2$ antagonist-induced catalepsy, and cocaine-induced rise of DARPP-32 Thr34 phosphoryla-

tion *in situ* in the striatum in $EP_1$-deficient mice. These findings together strongly suggest that $PGE_2$ functions as a critical modulator of the dopaminergic system in the striatum. Neurotransmitters such as glutamate, GABA, acetylcholine, and serotonin, neuromodulators such as adenosine, and neuropeptides such as opioids and cholecystokinin are known to regulate dopamine signaling in the striatum. However, the role of prostaglandins has been overlooked. Our present findings suggest that $PGE_2$–$EP_1$ signaling should be added to this growing list of regulators of dopaminergic signaling.

PGs including $PGE_2$ are produced from arachidonic acid released from the cell membrane by sequential catalysis of COXs and respective isomerases. There are two COX isoforms, COX-1, which is constitutively expressed, and COX-2, which is induced on demand. Berke et al. (1998) found that COX-2 expression is induced by $D_1$ receptor stimulation in the striatum treated with 6-hydroxydopamine, and suggested that this is part of a genetic adaptation mechanism associated with dopamine depletion. Given that COX-2 expression lasts over hours to days (Smith et al., 2000), the $PGE_2$–$EP_1$ signaling we found as a physiological regulator of dopamine signaling may function also as a long-term adaptive, paracrine-like mechanism to amplify dopamine signaling in the striatum under some pathological conditions.

Why has this seemingly very important modulatory action of $PGE_2$ been overlooked in the dopamine research field? One plausible reason is that COX inhibitors, such as aspirin and indomethacin, are generally without effects on dopaminergic functions such as motor control. These results suggest a possibility of other PG-dependent pathway(s) opposed to the $PGE_2$–$EP_1$ pathway operating in the striatum. Previously, Horton (1964) reported that intracerebroventricular administration of $PGE_2$ resulted in catalepsy in cats, and Schwarz et al. (1982) reported that intrastriatal injection of $PGE_2$ inhibited apomorphine-induced circling in mice. Interestingly, these $PGE_2$ actions appear to antagonize dopamine signaling, opposite to the facilitatory function of the $PGE_2$–$EP_1$ signaling we found here. This may indicate that other EP subtypes that potentially antagonize the effect of $EP_1$ are present in the striatum. The presence of such opposing pathways is seen in PGs working in other systems such as cardiovascular homeostasis and regulation of allergy (Kobayashi et al., 2004; Kunikata et al., 2005).

One of the unique characteristics of the $PGE_2$–$EP_1$ pathway is that this pathway modulates both $D_1$ and $D_2$ signaling positively. Given that there is a very low degree of overlap of $D_1$ and $D_2$ receptor in MSNs (Gerfen et al., 1990; Hersch et al., 1995), and that $EP_1$ is present both in $D_1$ and $D_2$ neurons, it is most likely that the $PGE_2$ acts directly on $EP_1$ of each neuron type, either $D_1$ or $D_2$, and augments its signaling. It is well known that $D_1$ and $D_2$ receptors have opposing actions on the activity of adenylyl cyclase: activation of $D_1$ receptors increases and that of $D_2$ receptors suppresses cAMP formation, and, consequently, Thr34 phosphorylation of DARPP-32 via PKA. Given that activation of $EP_1$ results in a rise in intracellular $Ca^{2+}$ ion (Watabe et al., 1993) and that a rise in intracellular $Ca^{2+}$ ion generally induces dephosphorylation of DARPP-32 Thr34 (Svenningsson et al., 2004), the effects of $EP_1$ stimulation on $D_1$ and $D_2$ signaling cannot be readily explained by their signal transduction. An indication as to the mechanism of $EP_1$ action is the fact that effects of the $EP_1$ deficiency can be overridden by increasing concentration of agonists (Figs. 3, 4, 6*D*) and that such an effect is seen also in the phenotype induced by an antagonist (Fig. 5). These findings suggest that $EP_1$ may modulate ligand-binding activity of $D_1$ and $D_2$ receptors. One possibility is that intracellular $Ca^{2+}$ increase might affect desensitization of $D_1$ and $D_2$ receptors by suppressing G-protein-coupled receptor kinases as suggested previously for the $D_1$ receptor (Tiberi et al., 1996; Iacovelli et al., 1999). An alternative possibility is that $EP_1$ receptors may modulate $D_1$ and $D_2$ ligand binding via receptor–receptor interaction. It is known that $D_2$ receptors (Fuxe et al., 1998; Rocheville et al., 2000) interact physically with other GPCRs such as the adenosine $A_{2A}$ receptor and the somatostatin SSTR5 receptor, and that heterooligomerization modulates ligand binding and signaling of the $D_2$ receptor. Similar oligomer formation may occur between $EP_1$ and $D_1$ and $EP_1$ and $D_2$ receptors to increase the binding affinities of the receptors for dopamine agonists and antagonists. This hypothesis may well explain the features of $EP_1$ action observed here. However, we failed to detect significant differences in binding properties of each type of dopamine receptor between wild-type and $EP_1$-deficient mice. Careful examination of the change of ligand binding activity using heterologous expression systems may reveal modulation of $D_1$ and $D_2$ receptor binding by $EP_1$.

Although we found in this study that $EP_1$ amplifies dopamine signaling, we previously reported that $EP_1$ deficiency increases dopamine turnover in the striatum and the frontal cortex, and that $EP_1$ apparently functions to suppress dopamine-dependent aggressive behavior (Matsuoka et al., 2005). In our preliminary work, we found that $EP_1$ facilitates GABAergic neurotransmission in substantia nigra pars compacta and negatively modulates the activity of dopaminergic neurons (our unpublished observation). $EP_1$ may also function in the orbitofrontal cortex and amygdala, the brain areas implicated in controlling impulsive aggression (Davidson et al., 2000). Localization of $EP_1$ in the latter was already reported (Matsuoka et al., 2003). $EP_1$ might therefore have distinct effects on multiple dopaminoceptive areas, each of which mediate unique motor control, emotional, or cognitive functions.

If $EP_1$ does indeed regulate dopaminergic signaling differently at different sites, it would offer a pharmacological means to modulate various dopaminergic functions in unique patterns. For instance, $EP_1$ agonists may augment dopaminergic activity only in the striatum, thus possibly circumventing psychotic symptoms associated with classical Parkinson therapy using levodopa (Factor et al., 1995). Similarly, treatment of schizophrenic patients with $EP_1$ agonists may suppress dopaminergic functions in the prefrontal cortex and limbic structures without extrapyramidal side effects, which still remain a common complication in antipsychotic therapy (Leucht et al., 2003). Our findings have not only demonstrated that the $PGE_2$–$EP_1$ pathway provides a modulatory mechanism of dopamine function in the striatum but also presented a novel strategic concept in development of drugs modulating dopaminergic pathways.

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