Characterization of Murine Vasopressor and Vasodepressor Prostaglandin E2 Receptors

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Abstract—Four E-prostanoid (EP) receptors, designated EP1, EP2, EP3, and EP4, mediate the cellular effects of prostaglandin E2 (PGE2). The present studies pharmacologically characterize the vasopressor and vasodepressor EP receptors in wild-type mice (EP2−/− mice) and mice with targeted disruption of the EP2 receptor (EP2−/− mice). Mean arterial pressure (MAP) was measured via a carotid artery catheter in anesthetized male mice. Intravenous infusion of PGE2 decreased MAP in EP2−/− mice but increased MAP in EP2−/− mice. Infusion of EP2-selective agonists, including MB28767, SC46275, and sulprostone, increased MAP in both EP2+/+ and EP2−/− mice. Pretreatment with SC46275 allowed a residual vasodepressor effect of PGE2 to be seen in the EP2−/− mice. An EP2-selective agonist (prostaglandin E1-OH) functioned also as a vasodepressor in both EP2−/− and EP2+/+ mice. High levels of EP3 receptor mRNA were detected in mouse aortas and rabbit preglomerular arterioles by nuclease protection, with lower expressions of EP1, EP2, and EP4 mRNA. The findings suggest that combined vasodepressor effects of EP2 and EP4 receptors normally dominate, accounting for the depressor effects of PGE2. In contrast, in EP2−/− mice, EP3 receptor activity alone is insufficient to overcome the EP2 vasopressor effect. These findings suggest that a balance between pressor and depressor PGE2 receptors determines its net effect on arterial pressure and that these receptors may be important therapeutic targets. (Hypertension. 2000;35:1129-1134.)

Key Words: hypertension, sodium-dependent ■ blood pressure ■ prostaglandins

Prostaglandins are critical regulators of vascular tone and arterial pressure.1–5 Inhibition of endogenous prostaglandin synthesis by NSAIDs may result in systemic hypertension or compromise the control of blood pressure in subjects with preexisting salt-sensitive hypertension.3,5 Prostaglandin E2 (PGE2) is a potent vasodilator in several vascular beds and directly relaxes preconstricted vascular rings.6 Conversely, PGE2 directly constricts smooth muscle in other tissues, including ileum and vas deferens.7,8 These opposing effects of PGE2 are thought to be mediated by distinct G-protein–coupled E-prostanoid (EP) receptors, designated EP1, EP2, EP3, and EP4.7 These receptors may be distinguished by their functional effects and by their affinity for structural analogues of PGE2. At least 2 constrictor EP receptors, designated EP1 and EP4, exist.7–11 The EP1 receptor signals through increased cell calcium and is highly active in vas deferens and ileal smooth muscle.7,8,12,13 The EP1 receptor signals through an inhibitory G protein (Gi) to decrease cAMP and is selectively activated by MB28767 and SC46275.11,14,15 There are also 2 distinct EP receptors that relax smooth muscle, designated EP2 and EP3.16,17 EP2 and EP3 receptors directly dilate vascular smooth muscle rings in vitro.16,17 Both these receptors increase cAMP but may be distinguished by their differential sensitivity to the EP2-selective agonist butaprost and the EP4-selective agonist prostaglandin E1 (PGE1)-OH.15,17 An important role for EP receptors in regulating systemic blood pressure has been suggested by recent studies in mice with targeted disruption of these receptors. Disruption of the EP2 receptor is associated with the development of salt-sensitive hypertension.18 Importantly, these mice also displayed an aberrant effect of PGE2 infusion on MAP, which increased blood pressure in EP2 knockout mice as opposed to the typical decrease in blood pressure observed in control mice.18 This finding suggests the importance of both pressor and depressor EP receptors in regulating systemic blood pressure. Recent studies have determined the affinity of prostaglandin analogues for all the cloned mouse prostanoid receptors, facilitating the interpretation of pharmacological studies examining the role of EP receptors in mice.15 The present studies were undertaken to pharmacologically characterize the pressor and depressor EP receptors mediating the hemodynamic effects of PGE2 infusion in the mouse.
Methods

Chemical Reagents
PGE₂ and PGE₁-OH were purchased from Biomol. Sulprostone was provided by Berlex Laboratories (Cedar Knolls, NJ). MB28767, which was generously provided by Michael Caton (Rhone-Poulenc Rorer, Dagenham, England), and SC46275, which was provided by Drs Richard Marks and Edward Drower (Searle R&D, Skokie, Ill), were dissolved in ethanol.

Animal Preparation
C57BL/6 mice were purchased from Harlan (Indianapolis, Ind). EP₂-deficient mice were developed as previously described. F₂ wild-type (EP₂⁺/⁺) and EP₂-null (EP₂⁻/⁻) mice were intercrossed, and EP₂-deficient mice were housed under controlled conditions (temperature 21°C, humidity 60±10%, lighting for 8 to 20 hours). Genotypes of mice were routinely determined by Southern analysis of genomic tail DNA. The wild-type (4.3-kb) and recombinant (7.5-kb) XbaI fragments were identified by using a 3'XbaI/SacI fragment as a probe.

Blood Pressure Measurement
Male F₂ EP₁⁺/⁺ and EP₁⁻/⁻ mice and C57BL/6 mice, age 12 to 16 weeks and 20 to 25 g in body weight, were anesthetized with 80 mg/kg ketamine (Fort Dodge Laboratories) and 8 mg/kg inactin (BYK) by intraperitoneal administration. Mice were placed on a temperature-controlled pad. After tracheostomy, PE-10 tubing was inserted into the right carotid artery, a jugular vein catheter was placed for infusion, and a urinary bladder catheter was inserted for urine collection. Blood pressure was measured with a Cobe CDX II transducer connected to a blood pressure analyzer (BPA 400, Micromed). The readings of blood pressure and heart rate were equilibrated for 30 to 60 minutes until stable values were obtained. The test agents (PGE₂, sulprostone, MB28767, SC46275, and PGE₁-OH) were mixed with 25 °C to 27°C. 1 ml of saline and injected as a bolus via the jugular vein over approximately 20 seconds. Blood pressures, including systolic, diastolic, and mean arterial pressure (MAP), and heart rates were recorded continuously on a thermal printer or computerized data record.

For measurement of blood pressure in conscious mice, we followed the technique of Mattson. Animals were preanesthetized with methoxyflurane and sodium pentobarbital (50 mg/kg IP). With use of an aseptic technique, femoral artery catheters were placed for MAP measurement, and femoral venous catheters were placed for infusion. Three days after surgery, PGE₂ (200 µg/kg) or SC46275 (20 µg/kg) was administered as a 100 µL bolus, and blood pressure was recorded by use of a Cobe CDX II transducer (described above).

Preparation of cDNA Probes
Mouse EP₁ (649-bp), EP₂ (420-bp), EP₃ (438-bp), and EP₄ (350-bp) cDNA fragments were generated by reverse transcription–polymerase chain reaction from mouse kidney and ileum total RNA and were cloned into pCR-TOPO vector (Invitrogen). Rabbit EP₁ (223-bp), EP₂ (345-bp), EP₃ (466-bp), and EP₄ (368-bp) cDNA fragments were also generated by reverse transcription–polymerase chain reaction from rabbit kidney and uterus and were also cloned into pCR-TOPO vector (Invitrogen). Mouse β-actin cDNA was obtained from Ambion Inc. Antisense and sense riboprobes were synthesized in vitro with the use of an appropriate RNA polymerase (Maxiscript, Ambion) and [³²P]UTP for RNase protection assays.

Solution Hybridization/RNase Protection Assay
RNase protection assays were performed as described previously. Briefly, plasmids containing rabbit or mouse EP₁, EP₂, EP₃, and EP₄, and rabbit GAPDH or mouse β-actin inserts, described above, were linearized with appropriate restriction enzymes. Radioactive riboprobes were synthesized from 1 µg of linearized plasmid in vitro by use of a Maxiscript kit (Ambion) for 1 hour at 37°C in a total volume of 20 µL. The reaction buffer contained 10 mmol/L dithiothreitol, 0.5 mmol/L ATP, CTP, and GTP, 2.5 mmol/L UTP, and 5 µL of 800 Ci/mmol [α-³²P]UTP at 10 mCi/mL (Dupont NEN). Hybridization buffer included 80% deionized formamide, 100 mmol/L sodium citrate, pH 6.4, and 1 mmol/L EDTA (RPA II, Ambion). Total RNA (20µg), isolated by Trizol-Reagent (GIBCO-BRL), was incubated at 45°C for 12 hours in hybridization buffer with 5×10⁵ cpm labeled riboprobes. After hybridization, ribonuclease digestion was carried out at 37°C for 30 minutes, and precipitated protected fragments were separated on 4% polyacrylamide gel at 200 V for 3 hours. The gel was exposed to Kodak XAR-5 film overnight at −80°C with intensifying screens.

Isolation of Renal Microvessels
Renal preglomerular microvessels were isolated by following the method of Chaudhari and Kirschenbaum. The renal artery of anesthetized New Zealand White rabbits was cannulated, and kidneys were perfused with 10 mL of ice-cold normal saline, followed by 10 mL of a 1% suspension of magnetized iron oxide (Fe₃O₄, Aldrich) in normal saline. The cortex of the kidneys was minced with a tissue press and homogenized with a Polytron homogenizer (Brinkmann) at moderate speed for 15 seconds twice. Microvessels were separated from nonvascular tissue in several washing steps (in 1× PBS) with the help of a strong magnet held to the outside of the tube. Washing and separation were repeated after passing the homogenate through 20-, 21-, and 23-gauge needles, respectively, until the suspension was mostly free of glomeruli and other nonvascular tissue. This technique provided a large quantity of relatively pure preglomerular microvessels, with 10% to 15% of the suspension consisting of attached glomeruli and small fragments of early proximal tubules.

Results

Different Effects of PGE₂ on Blood Pressure in Mice
The dose-dependent effect of PGE₂ on MAP pressure was determined in male mice (Table). Baseline MAPs were not significantly different in anesthetized EP₁⁺/⁺ and EP₁⁻/⁻ mice (108.5±5.2 [n=4] versus 104±19.2 [n=5], respectively). Sixty seconds after PGE₂ infusion, MAP was significantly decreased by 10.3±3.2 mm Hg in EP₁⁺/⁺ mice (Figure 1), whereas MAP was increased by 22.3±7.2 mm Hg in EP₁⁻/⁻ mice. These effects are qualitatively similar to those previ-
**EP3 Agonists Are Potent Vasopressors**

The dose-dependent effects of intravenous infusion of 3 EP3 receptor agonists, sulprostone (EP1>EP3), MB28767 (EP3), and SC46275 (EP3) on MAP were examined. As shown in the Table, all 3 EP3 agonists significantly increased MAP. Both MB28767 and SC46275 infusion (1 to 10 μg/kg) resulted in a prompt and marked increase in MAP with a progressive return to baseline over 15 minutes (Figure 2). Similarly, sulprostone, a pressor agonist that acts at the EP1 and EP3 receptors, increased MAP in wild-type and EP3−/− mice, as previously reported.18 Likewise the pressor effects of 2 EP3 agonists, MB28767 and SC46275, seen in EP3+/− mice were identical in EP3−/− mice (Table). These pressor effects are not specific for anesthetized mice because SC46275 (20 μg/kg) also increased MAP in conscious chronically catheterized mice, from 121.7±8.6 to 132.3±9.2 mm Hg (ΔMAP 10.7±1.2 mm Hg, n=6, P<0.0003), whereas PGE2 infusion (200 μg/kg) decreased MAP from 128.8±8.3 to 106.6±6.4 mm Hg (ΔMAP −22.2±6.2, n=5, P<0.02) in conscious mice.

**Desensitization of Pressor Effects With Use of EP3-Specific Agonist SC46275**

Experiments were designed to determine whether prior exposure to a pressor dose of an EP3-selective agonist desensitized mice to the subsequent effect of a second pressor agonist (Figure 3A). Pretreatment of mice with 10 μg/kg SC46275 transiently increased MAP as described above and completely prevented the subsequent pressor effect of sulprostone (20 μg/kg) on MAP. As also described above, EP3−/− mice characteristically show a pressor response to infusion of 100 μg/kg PGE2 (Figure 1); in contrast, wild-type animals react with a decrease in blood pressure. Prior desensitization with SC46275 (10 μg/kg) in EP3−/− mice changed the effect of PGE2 from a pressor to a depressor response (Figure 3B), suggesting the existence of a second depressor EP receptor, distinct from the EP1 receptor in mouse vasculature. Interestingly, the depressor response observed after desensitization was significantly greater in EP2−/− mice than in EP3−/− mice (ΔMAP −35.6±1.7 mm Hg [EP2−/−, n=4] versus −18.3±3.7 mm Hg [EP3−/−, n=4], P<0.01 by ANOVA).

**EP4 Receptor Agonist PGE1-OH Is Vasodepressor**

To pharmacologically characterize and determine whether this depressor receptor could be activated in mice without prior desensitization, the effect of an EP4-selective agonist, PGE1-OH, was examined. In contrast to the pressor effects of native PGE2 in EP3−/− mice, intravenous administration of PGE1-OH decreased MAP in both wild-type and EP3−/− mice to a similar extent (Table and Figure 4). At a dose of 100 μg/kg PGE1-OH, infusion resulted in a prompt fall in MAP, which was maximal within 1 minute and then gradually returned to baseline. The maximal decrease in MAP was not significantly different in wild-type mice and EP3−/− mice.

**Expression of EP Receptor mRNA in Blood Vessels**

RNase protection assays were performed to determine the relative expression of mRNA for the 4 EP receptors in mouse aorta and rabbit preglomerular renal vessels. Expression of the EP3 receptor mRNA predominated in both vessels, exhibiting a much stronger signal than the other potential constrictor receptor, the EP1 receptor (Figure 5). Nuclease protections of mRNA from rabbit preglomerular vessels were repeated on 3 separate preparations. Densitometry normalized to GAPDH demonstrates that rabbit EP1 receptor mRNA is 7.7±0.7-fold more abundant than rabbit EP2 receptor mRNA and 47.8±3.8-fold more abundant than EP2 receptor mRNA (both

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**Figure 1.** Effects of PGE2 infusion on MAP in male EP2+/+ (□) and EP2−/− (○) mice. PGE2 (100 μg/kg) was infused intravenously at time=0 seconds as described in Methods. *P<0.05 vs baseline MAP (paired t test). MAP did not change in mice receiving vehicle alone (not shown).

**Figure 2.** Effects of 2 EP3 agonists, SC46275 and MB28767, on MAP in anesthetized EP2+/+ mice. Mice received bolus administration of either SC46275 or MB28767 (10 μg/kg IV, n=5) at time=0 seconds (arrow) as described in Methods. Each point within the bracket is significantly greater than its respective baseline value.
A similar pattern of expression was observed in mouse aortas.

Discussion

Accumulating evidence supports an important role for vasodilator prostanoids in preventing hypertension in humans. Control of blood pressure in patients with essential hypertension is particularly dependent on endogenous vasodilator prostaglandins. In mice with targeted disruption of the angiotensin type 2 receptor, increased synthesis of dilator prostaglandins, including PGE2, helps maintain normotension, and these mice develop hypertension when treated with an NSAID. The prostanoid receptors maintaining normotension and mediating these dilator responses have been only partially characterized. Targeted disruption of the EP2 receptor is associated with the development of salt-sensitive hypertension in EP2−/− but not wild-type mice. These studies also found that the normal vasodepressor effect of the EP2-selective agonist butaprost was absent in EP2−/− mice. Furthermore, when PGE2, an endogenous ligand, was infused in EP2−/− mice, it increased arterial pressure rather than producing the typical vasodepressor effect observed in EP2+/+ mice. These findings point to the existence of the regulation of blood pressure by multiple EP receptors. The present studies were designed to further characterize the prostaglandin E receptor subtypes regulating arterial pressure in the mouse.

Initial studies confirmed that PGE2 infusion increased mean arterial pressure in male EP2−/− mice in a manner similar to that previously reported for female EP2−/− mice. Conversely, PGE2 functioned as a vasodepressor in male EP2+/+ mice. Thus, disruption of the EP2−/− receptor converts the dominant effect of PGE2 from a vasodepressor to a vasopressor. Some studies have suggested that the female circulation exhibits greater sensitivity to the hemodynamic effects of prostaglandins than does the male circulation. The present findings suggest that this possibility holds true for the effect of PGE2 on MAP, because there was a tendency for the depressor effect of PGE2 in male EP2+/+ mice to be less than in female EP2+/+ mice (ΔMAP after PGE2 was −10.3±3.2 mm Hg in males versus −19.2±5.2 mm Hg in females). However, this trend did not achieve statistical significance. Further studies are required to determine whether gender influences the extent of these changes. Regardless of these considerations, the qualitative effect of PGE2 on MAP appears similar in female and male EP2+/+ mice, in which it acts as a vasodilator, compared with EP2−/− mice, in which it acts as a vasopressor.
The present studies found a potent pressor effect of 2 highly selective EP agonists, MB28767 and SC46275. SC46275 is $10^5$-fold more potent at EP receptors than at EP receptors, activating EP receptors in ileal smooth muscle only at concentrations $\approx 30 \mu$mol/L, whereas sulprostone comparably activates EP receptors at 20 nmol/L. The potent vasopressor effect of SC46275 suggests an EP, rather than an EP-mediated effect. Similar findings with MB28767 support this conclusion. Molecular characterization of all the cloned murine prostanoid receptors show that MB28767 binds the EP receptor 200-fold more avidly than the EP receptor or any other prostanoid receptor. These findings confirm and extend previous studies with the EP, selective agonist, sulprostone, and point to a specific role for the EP receptor in regulating vascular tone.

The 3 EP agonists used also appear to act via a common mechanism, because pretreatment with an EP-selective agonist prevented the pressor response to a second EP agonist (Figure 3A). The precise mechanism of desensitization remains uncertain but could be consistent with studies showing agonist-induced desensitization and internalization of the cloned mouse EP receptor expressed in COS cells. Alternatively, the persistence of the EP agonist (SC46275) in the circulation because of poor metabolic clearance could lead to ongoing receptor activation.

Regardless of the desensitization mechanism, pretreatment with EP-selective analogues did not block the subsequent vasodepressor effect of PGE, supporting a distinct mechanism for this effect (Figure 3B). It is of note that after SC46275 “desensitization” of the vasopressor EP-prostanoid response, a remaining vasodepressor effect of PGE in EP mice was revealed. This supports the persistence of a separate vasodepressor EP receptor in EP mice, possibly the EP receptor. This conclusion is further supported by the observation that even without prior desensitization, PGE, an EP-selective agonist, was a depressor in EP mice. Interestingly, after pretreatment with SC46275, the vasodepressor effect of PGE, is significantly greater in EP mice than in EP mice, consistent with the possibility that PGE activates both EP and EP depressor receptors in EP mice but only the EP receptor in EP mice.

Multiple mechanisms undoubtedly contribute to the effects of PGE on blood pressure in the intact animal, including effects on sympathetic nervous activity and renin release and direct effects on vascular tone. The present studies used nuclease protection to demonstrate that EP receptor mRNA is expressed in mouse and rabbit vasculature. These studies demonstrate that EP receptor mRNA was expressed at significantly higher levels than the EP, EP, and EP receptor mRNAs. Although it is uncertain whether this EP receptor mRNA expression pattern parallels receptor density, these findings support the results of functional studies suggesting roles for the EP, EP, and EP receptors in modulating blood pressure. Furthermore, the presence of EP receptor mRNAs in the vasculature suggests that direct effects on vascular tone may contribute to the observed effects of PGE infusion on MAP. They additionally support a specific role for the EP receptor as a vasopressor receptor in rabbit renal resistance arterioles.

In summary, the present studies provide evidence that the net effect of PGE infusion on blood pressure results from a balance between the functional activity of at least 3 distinct EP receptors. Data using an EP-selective analogue and EP mice support independent roles for EP and EP receptors as vasodepressors in the mouse circulation. These findings also suggest a critical role the EP receptor as an important vasopressor receptor. No functional evidence supports an independent role for the EP receptor as a vasopressor. Given the importance of prostaglandin E receptors in the regulation of blood pressure, selective agonists and antagonists of vasoactive EP receptors may provide important new therapeutic targets for the treatment of hypertension.

**Note Added in Proof**

After this manuscript was submitted, another study documenting the role of EP receptors as vasopressors and vaso-
depressors in knockout mice was published by Audoly et al (Am J Physiol. 1999;277:H924–H930).

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