Prostaglandin I\textsubscript{2} Contributes to the Vasodepressor Effect of Baicalein in Hypertensive Rats

Hideki Takizawa, AnnMarie DelliPizzi, Alberto Nasjletti

Abstract—Lipoxygenase inhibitors reduce blood pressure in hypertensive rats. The vasodepressor effect of lipoxygenase inhibitors may be related to increased production of prostaglandin (PG) I\textsubscript{2} since lipoxygenase-derived fatty acid hydroperoxides inhibit PGI\textsubscript{2} synthase. This hypothesis was examined in rats made hypertensive by infusion of angiotensin II (200 ng/min IP) for 12 to 14 days. In hypertensive but not in normotensive rats, the lipoxygenase inhibitor baicalein (60 mg/kg SC) increased (P<.05) the conversion of exogenous PGH\textsubscript{2} to PGI\textsubscript{2} by aortic segments, the release of 6-keto-PGF\textsubscript{1α}, by aortic rings, the concentration of 6-keto-PGF\textsubscript{1α} in blood, and the renal excretion of 6-keto-PGF\textsubscript{1α}. Treatment with baicalein did not affect the blood pressure of normotensive rats but decreased the blood pressure of hypertensive rats from 177±8 to 133±9 mm Hg after 120 minutes (P<.05). Also, the lipoxygenase inhibitor cinnamyl-3,4-dihydroxy-α-cyanocinnamate (8 mg/kg SC) was without effect on the blood pressure of normotensive rats but decreased the blood pressure of hypertensive rats from 182±4 to 139±8 mm Hg (P<.05). However, the blood pressure of hypertensive rats pretreated with indomethacin (5 mg/kg IV) was affected by neither baicalein nor cinnamyl-3,4-dihydroxy-α-cyanocinnamate. Moreover, in hypertensive rats in which baicalein had decreased blood pressure to 148±6 mm Hg, the administration of rabbit serum containing antibodies against 5,6-dihydro-PGI\textsubscript{2} (0.3 mL IV) partially reversed the response to baicalein, increasing blood pressure to 179±7 mm Hg within 20 minutes (P<.05). The antibodies also were shown to block the vasodepressor effect of PGI\textsubscript{2} but not of PGE\textsubscript{2}. Collectively, these data suggest contribution of PGI\textsubscript{2} to the acute antihypertensive effect of baicalein in rats with angiotensin II–induced hypertension. (Hypertension. 1998;31:866-871.)

Key Words: angiotensin II ■ prostaglandin I\textsubscript{2} ■ prostacyclin synthase ■ 12-lipoxygenase ■ lipoxygenase inhibitors

Vascular tissues contain a lipoxygenase that catalyzes the oxygenation of C12 of arachidonic acid.\textsuperscript{1,2} The product of this reaction is 12-HPETE, which undergoes spontaneous or peroxidase-catalyzed reduction to 12-HETE.\textsuperscript{4} Both 12-HPETE and 12-HETE are capable of influencing vascular functions. 12-HPETE inhibits vascular PGI\textsubscript{2} synthase activity\textsuperscript{3} and was reported to increase the expression of arachidonic acid–induced, PGH\textsubscript{2}-mediated constrictor responses in rings of rat aorta.\textsuperscript{6} 12-HETE was shown to depolarize renal arterial smooth muscle cells,\textsuperscript{7} increase the protein content of cultured porcine aortic smooth muscle cells,\textsuperscript{8} and facilitate the stimulatory actions of Ang II and vasopressin on calcium transients in cultured smooth muscle.\textsuperscript{9}

Several studies indicate that Ang II promotes lipoxygenase-catalyzed production of eicosanoids in vascular tissue. For example, Ang II was reported to stimulate release of 12-HETE from segments of human umbilical artery\textsuperscript{10} and to increase 12-lipoxygenase-derived HETEs by segments of thoracic aorta.\textsuperscript{8} The conversion of exogenous PGH\textsubscript{2} to PGI\textsubscript{2} by aortic segments, the release of 6-keto-PGF\textsubscript{1α} by aortic rings, the concentration of 6-keto-PGF\textsubscript{1α} in blood, and the renal excretion of 6-keto-PGF\textsubscript{1α}. Treatment with baicalein did not affect the blood pressure of normotensive rats but decreased the blood pressure of hypertensive rats from 182±4 to 139±8 mm Hg (P<.05). However, the blood pressure of hypertensive rats pretreated with indomethacin (5 mg/kg IV) was affected by neither baicalein nor cinnamyl-3,4-dihydroxy-α-cyanocinnamate. Moreover, in hypertensive rats in which baicalein had decreased blood pressure to 148±6 mm Hg, the administration of rabbit serum containing antibodies against 5,6-dihydro-PGI\textsubscript{2} (0.3 mL IV) partially reversed the response to baicalein, increasing blood pressure to 179±7 mm Hg within 20 minutes (P<.05). The antibodies also were shown to block the vasodepressor effect of PGI\textsubscript{2} but not of PGE\textsubscript{2}. Collectively, these data suggest contribution of PGI\textsubscript{2} to the acute antihypertensive effect of baicalein in rats with angiotensin II–induced hypertension. (Hypertension. 1998;31:866-871.)

Key Words: angiotensin II ■ prostaglandin I\textsubscript{2} ■ prostacyclin synthase ■ 12-lipoxygenase ■ lipoxygenase inhibitors

Received August 25, 1997; first decision September 22, 1997; revision accepted October 31, 1997.

from the Department of Pharmacology, New York Medical College, Valhalla, NY. Correspondence to Alberto Nasjletti, MD, Department of Pharmacology, New York Medical College, Valhalla, NY 10595. © 1998 American Heart Association, Inc.
the abdominal cavity an osmotic minipump filled with 0.01 mol/L acetic acid, the vehicle of Ang II.

Animals in protocols 1, 2, and 3 were instrumented with a chronic arterial catheter. One day before minipump placement, rats were anesthetized with pentobarbital sodium (60 mg/kg IP) and a polyethylene cannula (PE-50) filled with saline solution (0.15 mol/L NaCl) containing heparin (100 units/mL) was introduced through the left femoral artery and advanced into the lower abdominal aorta. Animals in protocols 2 and 3 also were fitted with a venous cannula (PE-50) introduced through the left femoral vein and advanced into the lower inferior vena cava. Both cannulas were tunneled subcutaneously to an exit point at the nape of the neck and plugged with steel pins until use. All rats received ampicillin (30 mg · kg⁻¹ · 12 h⁻¹ SC) for 3 days after surgery.

Experiments were conducted 12 to 14 days after minipump placement. In protocols 1, 2, and 3, the mean arterial pressure of awake rats was measured via the femoral arterial cannula by means of a pressure transducer (model P23XL; Statham Division, Gould Inc) coupled to a polygraph (model 7D; Grass Instrument). In protocol 4, systolic blood pressure was determined through tail sphygmomanometry with the use of an electrosphygmomanometer (Narco Bio-System).

**Experimental Protocols**

Protocol 1 was designed to examine the effect on blood pressure of treatment with lipooxygenase inhibitors baicalein or CDC₆.¹²,¹⁴,¹⁸ (BIOLUML Research Laboratories). On the day of the experiment, 12 to 14 days after minipump placement, rats with Ang II–induced hypertension were injected subcutaneously with baicalein (60 mg/kg; n = 8), CDC (8 mg/kg; n = 9), or vehicle (sesame oil, 2.0 mL/kg of body wt; n = 11). Sham-infused normotensive rats also were injected with baicalein (60 mg/kg SC; n = 6), CDC (8 mg/kg SC; n = 4), or sesame oil vehicle (2.0 mL/kg of body wt; n = 4). Mean arterial pressure was monitored before and after treatment.

Protocol 2 was designed to investigate the contribution of vasodepressor prostanoids to the effects of baicalein and CDC on blood pressure of rats with Ang II–induced hypertension. Hypertensive rats were pretreated intravenously with indomethacin (5 mg/kg bolus injection followed by infusion at 5 mg/kg per hour) or drug/vehicle alone. Sixty minutes later, baicalein was administered subcutaneously (60 mg/kg) to animals pretreated with indomethacin (n = 6) or vehicle alone (n = 8), and blood pressure was monitored over the next 120 minutes. Likewise, rats pretreated with indomethacin (n = 7) or drug/vehicle alone (n = 6) were injected subcutaneously with CDC (8 mg/kg), and blood pressure was recorded.

Protocol 3 was designed to investigate the contribution of PGI₂ to the effect of baicalein on blood pressure of rats with Ang II–induced hypertension. Hypertensive rats were injected subcutaneously with baicalein (60 mg/kg), followed 60 minutes later by an intravenous injection of nonimmune rabbit serum (0.3 mL; n = 5) or rabbit serum containing antibodies directed against 5,6-dihydro-PGI₂ (0.3 mL; n = 5). Blood pressure was monitored throughout the experiment. The serum containing 5,6-dihydro-PGI₂ antibodies was donated by Dr Lawrence Levine (Brandeis University). The method of immunization against 5,6-dihydro- PGI₂ and the characteristics of the resulting antibodies were described previously.²⁰,²¹ 5,6-Dihydro-PGI₂ antibodies were reported to bind PGI₂ and neutralize the vasodepressor action of PGI₂.²⁰,²¹

We conducted complementary studies to validate the effectiveness and specificity of 5,6-dihydro-PGI₂ antiserum to block the vasodepressor effect of PGI₂ in rats. Experiments were conducted in awake Sprague-Dawley rats previously instrumented with femoral arterial and venous cannulas to measure blood pressure and administer drugs, respectively. The effect of intravenous bolus injections of PGI₂ (2.0 µg/kg) on blood pressure was examined before and at intervals after intravenous administration of 5,6-dihydro-PGI₂ antiserum (0.3 mL; n = 5). In separate rats, the effect of intravenous bolus injections of PGE₂ (2.0 µg/kg) on blood pressure was examined before and at intervals after intravenous administration of 5,6-dihydro-PGI₂ antiserum (0.3 mL; n = 5).

Protocol 4 was designed to investigate the effect of in vivo treatment with baicalein on release of 6-keto-PGF₁α from rings of descending thoracic aorta, conversion of exogenous PGI₂ to PGI₂ by rings of descending thoracic aorta, concentration of prostanoids in venous blood, and renal excretion of 12-HETE and prostanoids. On the day of the experiment, sham-infused rats and rats with Ang II–induced hypertension were injected with baicalein (n = 6) or vehicle only (n = 6) as described in protocol 1. One hour later, the rats were anesthetized with pentobarbital sodium (60 mg/kg IP), the abdominal cavity was exposed through a midline incision, the inferior vena cava was punctured with an 18-gauge needle to sample blood (1 mL) for measurement of prostanoids, and the descending thoracic aorta was excised and cut into ring segments (3 mm in length). The aortic rings were used immediately to assess release of 6-keto-PGF₁α and ability to convert exogenous PGI₂ to PGI₂.

In additional experiments, rats with Ang II–induced hypertension of 12 days’ duration were housed in metabolic cages and subsequently injected with baicalein (n = 8) or vehicle only (n = 8) as described in protocol 1. Thereafter, urine was collected for 3 hours to measure renal excretion of prostanoids and 12-HETE. Similar experiments were conducted in sham-infused normotensive rats injected with baicalein (n = 6) or vehicle (n = 7).

**Analytical Procedures**

**Measurement of 6-Keto-PGF₁α Release by Rings of Thoracic Aorta**

Aortic rings were incubated in Krebs’ bicarbonate buffer (2.0 mL) containing arachidonic acid (10 µmol/L) for 15 minutes at 37°C in an atmosphere of 95% O₂/5% CO₂. The amount of 6-keto-PGF₁α, in the medium, an estimate of PGI₂ release, was analyzed as previously described through enzyme immunoassay of unextracted samples with reagents purchased from Cayman Chemical.²² The results are expressed as picromoles of 6-keto-PGF₁α released during the 15-minute incubation period per milligram of dry tissue. 6-Keto-PGF₁α could not be detected in samples generated through incubation of aortic rings denatured by heating at 100°C for 5 minutes.

**Measurement of Conversion of PGH₂ to PGI₂ by Rings of Thoracic Aorta**

Aortic rings were preincubated for 20 minutes at 37°C in Krebs’ bicarbonate buffer gassed with 95% O₂/5% CO₂ and containing indomethacin (10 µmol/L) to inhibit cyclooxygenase.¹ The rings were then transferred to 20-mL vials containing fresh buffer (2.0 mL) for incubation at 37°C for 5 minutes in the presence and absence of exogenous PGH₁ (1 µmol/L). The concentration of 6-keto-PGF₁α, in the incubation media was measured through enzyme immunoassay of unextracted samples.²² The concentration of 6-keto-PGF₁α in medium derived from incubation of aortic rings in indomethacin-containing buffer without exogenous PGH₁ was <3% of the concentration in medium derived from incubations carried out in the presence of exogenous PGH₁. Hence, ≥97% of the 6-keto-PGF₁α in the incubation medium with PGH₁ arises from exogenous PGH₁. Results of the conversion of PGH₁ to PGI₂ are expressed as picromoles of 6-keto-PGF₁α formed during a 3-minute incubation per milligram of dry tissue. No conversion of exogenous PGH₁ to PGI₂ could be detected in control incubations using aortic rings denatured by heating at 100°C for 5 minutes. Hence, when cyclooxygenase is inhibited by
indomethacin, the conversion of exogenous PGH₂ to PGI₂ by aortic rings reflects the tissue activity of PGI₂ synthase.

**Measurement of PGE₂ and 6-Keto-PGF₁α in Blood**

Blood (1 mL) was drawn from the inferior vena cava into a syringe containing 4 mL of ice-cold ethanol and indomethacin (10 μg/mL). The mixture was stored at −20°C for 24 hours, followed by centrifugation at 1500 g for 10 minutes, evaporation of the supernatant under a stream of nitrogen, and reconstitution of the residue in 2 mL of 0.1 mol/L formic acid. PGE₂ and 6-keto-PGF₁α in the formic acid solution were further purified with passage through a column of octadecylsilyl silica (Sep-Pak C-18 cartridges; Waters Associates) according to a published procedure, followed by quantification through enzyme immunoassay. The results are expressed as picomoles of eicosanoid per 3 hours.

**Measurements of Eicosanoids in Urine**

The contents of PGE₂, 6-keto-PGF₁α, and 12-HETE in 3-hour urine samples were determined after purification with passage of the samples through a column of octadecylsilica. PGE₂ and 6-keto-PGF₁α were measured with enzyme immunoassay. 12-HETE was measured through radioimmunoassay using reagents and a protocol provided by PerSeptive Diagnostics. Data on urinary excretion of eicosanoids are expressed as picomoles of eicosanoid per 3 hours.

**Statistical Analysis**

Results are expressed as mean±SEM. ANOVA followed by the Newman-Keuls posteriori test was applied to the analysis of data on the effect of drugs on blood pressure and for comparisons among rats with Ang II–induced hypertension and sham-infused controls. Data on the effect of baicalein on 6-keto-PGF₁α release from aortic tissue, blood level of prostaglandins, urinary eicosanoid excretion, and vascular conversion of PGH₂ to PGI₂ were analyzed with unpaired Student's t test. The null hypothesis was rejected at a level of P<.05.

**Results**

Fig 1 displays data on mean arterial pressure before and after the administration of baicalein or sesame oil vehicle only to sham-infused normotensive rats and rats with Ang II–induced hypertension of 12 to 14 days’ duration. Before treatment, blood pressure was 103±8 mm Hg and 177±8 mm Hg (P<.05) in sham-infused rats and Ang II–infused rats, respectively. Treatment with baicalein caused blood pressure to fall progressively in rats with Ang II–induced hypertension, reaching a level of 133±9 mm Hg (P<.05) after 120 minutes. In contrast, treatment with baicalein was without effect on blood pressure in sham-infused normotensive rats. Likewise, treatment with CDC did not affect the blood pressure of sham-infused normotensive rats (103±8 mm Hg before and 120 minutes after CDC, respectively) but decreased (P<.05) the blood pressure of rats with Ang II–induced hypertension from 182±4 to 142±10 and 139±8 mm Hg after 60 and 120 minutes, respectively. The administration of drug/vehicle only did not affect the blood pressure of sham-infused normotensive rats or of rats with Ang II–induced hypertension.

The effects of baicalein on mean arterial pressure in rats with Ang II–induced hypertension pretreated and not pretreated with indomethacin are shown in Fig 2. Before baicalein administration, blood pressure was comparable in hypertensive rats pretreated and not pretreated with indomethacin. Like baicalein, the administration of CDC did not affect the blood pressure of hypertensive rats pretreated with indomethacin. In contrast, treatment with baicalein had little or no effect on the blood pressure of hypertensive rats pretreated with indomethacin. Like baicalein, the administration of CDC did not affect the blood pressure of hypertensive rats pretreated with indomethacin (176±8 mm Hg) and not pretreated (176±8 mm Hg) with indomethacin. Treatment with baicalein decreased (P<.05) blood pressure to 132±11 mm Hg in hypertensive rats without indomethacin pretreatment. In contrast, treatment with baicalein had little or no effect on the blood pressure of hypertensive rats pretreated with indomethacin. Like baicalein, the administration of CDC did not affect the blood pressure of hypertensive rats pretreated with indomethacin (176±5 and 177±5 mm Hg before and 120 minutes after CDC, respectively) but decreased (P<.05) the blood pressure of hypertensive rats without indomethacin pretreatment from 184±5 to 138±13 and 144±8 mm Hg after 60 and 120 minutes, respectively.

Fig 3 shows a comparison of the effects of nonimmune serum and 5,6-dihydro-PGI₂ antiserum on the blood pressure of rats with Ang II–induced hypertension that were pretreated with indomethacin or PGE₂ (2.0 μg/kg) or PGE₂ (2.0 μg/kg) on the mean arterial pressure of normotensive rats before and after the intravenous administration of 5,6-dihydro PGI₂ antiserum in Fig 4. Before
the injection of the antiserum, PG\textsubscript{I\_2} and PGE\textsubscript{2} caused blood pressure to fall promptly by 36 ± 6 and 22 ± 2 mm Hg, respectively, followed by a return to preinjection levels within the next 3 to 4 minutes. After injection of the antiserum, vasodepressor responsiveness to PG\textsubscript{I\_2} but not to PGE\textsubscript{2} was attenuated (P < .05) for up to 90 minutes.

Fig 5 displays data on the conversion of exogenous PG\textsubscript{H\_2} to PG\textsubscript{I\_2} by rings of descending thoracic aorta taken from normotensive and hypertensive rats with and without baicalein treatment. In animals without baicalein treatment, the conversion of PG\textsubscript{H\_2} to PG\textsubscript{I\_2} by aortic rings of normotensive rats surpassed (P < .05) that by aortic rings of hypertensive rats. Baicalein treatment of the hypertensive rats increased (P < .05) the conversion of exogenous PG\textsubscript{H\_2} to PG\textsubscript{I\_2} by aortic rings, whereas baicalein treatment of normotensive rats did not.

Fig 6 shows data on the release of 6-keto-PGF\textsubscript{1\_a} from the rings of descending thoracic aorta taken from normotensive and hypertensive rats with and without baicalein treatment. In animals without baicalein treatment, the release of 6-keto-PGF\textsubscript{1\_a} from aortic rings incubated in medium containing arachidonic acid was higher (P < .05) in hypertensive than in normotensive rats. Baicalein treatment of the hypertensive rats increased further (P < .05) the release of 6-keto-PGF\textsubscript{1\_a} from aortic rings, whereas baicalein treatment of normotensive rats was without effect.

The Table displays data on blood prostaglandins and urinary excretion of eicosanoids in normotensive and hypertensive rats with and without baicalein treatment. In animals without baicalein treatment, the blood concentration of 6-keto-PGF\textsubscript{1\_a} and the urinary excretion rate of 6-keto-PGF\textsubscript{1\_a} and 12-HETE were higher (P < .05) in hypertensive than in normotensive rats. Baicalein treatment of the hypertensive rats increased further (P < .05) the blood concentration and urinary excretion of 6-keto-PGF\textsubscript{1\_a} while decreasing (P < .05) the blood concentration of PGE\textsubscript{2} and the urinary excretion of 12-HETE. Baicalein treatment of normotensive rats did not modify the blood concentration and urinary excretion of 6-keto-PGF\textsubscript{1\_a} and PGE\textsubscript{2} but tended to decrease 12-HETE urinary excretion.

Discussion

The results of the present study demonstrate that treatment with baicalein or CDC lowers blood pressure in rats with Ang II–induced hypertension but not in normotensive rats. The study also demonstrates that the renal excretion of 12-HETE is greater in hypertensive than in normotensive rats and that baicalein treatment of the hypertensive rats reduces urinary

Figure 3. Effect of 5,6-dihydro-PGI\textsubscript{2} antiserum (●, 0.3 mL IV, n=5) or nonimmune serum (○, 0.3 mL IV, n=5) on mean arterial pressure of rats with Ang II–induced hypertension pretreated with baicalein (60 mg/kg SC). Results are mean±SEM; n indicates number of rats. *P < .05 relative to data obtained just before the injection of antiserum or nonimmune serum.

Figure 4. Reductions in mean arterial pressure (MAP) induced by bolus injections of PG\textsubscript{I\_2} (2.0 μg/kg IV, n=5; top) or PGE\textsubscript{2} (2.0 μg/kg IV, n=5; bottom) in normotensive rats before (open bars) and after (filled bars) the administration of 5,6-dihydro-PGI\textsubscript{2} antiserum (0.3 mL IV). Results are mean±SEM; n indicates number of rats. **P < .05 relative to control data obtained before antiserum administration.

Figure 5. Conversion of exogenous PG\textsubscript{H\_2} to PG\textsubscript{I\_2} (measured as 6-keto-PGF\textsubscript{1\_a}) by rings of descending thoracic aorta during incubation for 3 minutes at 37°C in indomethacin-containing Krebs’ bicarbonate buffer. The aortic rings were obtained from sham-infused normotensive rats and rats with Ang II–induced hypertension treated with baicalein (60 mg/kg SC) or sesame oil vehicle only. Results are mean±SEM; n indicates number of rats. *P < .05 relative to data in vehicle-treated rats.

Figure 6. Release of 6-keto-PGF\textsubscript{1\_a} from rings of descending thoracic aorta incubated for 15 minutes at 37°C in Krebs' bicarbonate buffer containing arachidonic acid. Aortic rings were obtained from sham-infused normotensive rats and rats with Ang II–induced hypertension treated with baicalein (60 mg/kg SC) or sesame oil vehicle. Results are mean±SEM; n indicates number of rats. *P < .05 relative to data in vehicle-treated rats.
12-HETE excretion to levels not different from those in normotensive rats, which is in agreement with reports that the drug inhibits lipooxygenase(s).6,12,18 These findings are consistent with involvement of products of lipooxygenase activity in the mechanisms underlying Ang II–dependent hypertension in rats. This conclusion also receives support from reports that Ang II promotes vascular expression of 12-lipoxygenase,1 tissue production of lipooxygenase products is increased in models of Ang II–dependent hypertension,6,11 and lipooxygenase-derived eicosanoids contribute directly or indirectly to the vascular actions of Ang II.5,9,10,12

The acute antihypertensive effect of baicalein and CDC in rats with Ang II–induced hypertension may be a functional consequence of diminished production of lipooxygenase–derived eicosanoids that mediate or facilitate vasoconstrictor mechanisms.6,7 It also may be linked to activation of a vasodilatory mechanism mediated by PGI2 and/or to deactivation of a pressor mechanism mediated by PGH2, both of which are events caused by elimination of the inhibitory influence of 12-HPETE and other lipooxygenase products on prostacyclin synthase.5,6,15,16 This study demonstrates that baicalein and CDC do not reduce the blood pressure of hypertensive rats pretreated with indomethacin. Because indomethacin inhibits cyclooxygenase without affecting the vascular production of lipooxygenase–derived HETEs,6 our results suggest that the acute antihypertensive effect of these agents in rats with Ang II–induced hypertension relies for its implementation on a prostanooid-mediated mechanism. This conclusion derives additional support from experiments demonstrating that the vasodepressor effect of baicalein in hypertensive rats is reversed partially through treatment with 5,6-dihydro-PIG1 antiserum. The administration of 5,6-dihydro-PIG1 antiserum also attenuates vasodepressor responsiveness to PGI2 but not to PGE2, which is in agreement with a report that antibodies directed to 5,6-dihydro-PIG1 bind and neutralize the biological activities of PGI2.20,21 The observation that 5,6-dihydro-PIG1 antiserum causes partial reversal of the antihypertensive effect of baicalein implicates PGI1 in the implementation of such an effect.

Previous reports show that vascular and renal production of PGI2 are increased in rats with Ang II–dependent hypertension.17 In concordance with such studies, we found that rats made hypertensive through infusion of Ang II feature increased circulating levels of 6-keto-PGF1α, elevated urinary excretion of 6-keto-PGF1α, and enhanced release of 6-keto-PGF1α from rings of thoracic aorta during incubation in medium containing arachidonic acid. Paradoxically, the ability of aortic rings to metabolize exogenous PGH1 to PGI2 was reduced in rats with Ang II–induced hypertension, implying that vascular PGI2 synthase activity is reduced in the hypertensive rats. In these animals, the increased release of 6-keto-PGF1α from aortic rings may be linked to overproduction of PGH1α, with the resulting elevation in cellular PGH1 concentration driving up PGI2 production in the face of reduced PGI2 synthase activity.

A major finding in this study is that the antihypertensive effect of baicalein in rats with Ang II–induced hypertension is accompanied by an increase in the rate of conversion of exogenous PGH1 to PGI1 by aortic rings, bringing it up to the rate of conversion found in aortic rings from normotensive rats. Previously, it was reported that rings of thoracic aorta taken from rats with aortic coarctation–induced hypertension are impaired in their ability to convert exogenous PGH1 to PGI1 and that this impairment is corrected through exposure of the rings to baicalein or CDC.6 The notion that baicalein promotes metabolism of PGH1 to PGI1 in models of Ang II–dependent hypertension fits well with our findings that baicalein treatment of hypertensive rats increases the release of 6-keto-PGF1α from aortic segments incubated in medium containing arachidonic acid, the concentration of 6-keto-PGF1α in blood, and the renal excretion of 6-keto-PGF1α. These effects of baicalein are not driven by mechanisms that promote formation of all prostanoids because baicalein treatment of hypertensive rats elicited reduction of blood PGE2 levels and did not affect the renal excretion of PGE2.

It is unlikely that baicalein stimulates PGI1 synthase directly because its administration to normotensive rats did not increase the conversion of PGH1 to PGI1 by aortic rings or increase the release of 6-keto-PGF1α from aortic rings incubated with arachidonic acid, the blood concentration of 6-keto-PGF1α, or the renal excretion of 6-keto-PGF1α. The effects of baicalein on the status of PGI1 formation and levels in rats with Ang II–induced hypertension may be the result of interference with the production of endogenous factors capable of disrupting the metabolism of PGH1 to PGI1 by PGI1 synthase. In this context, reports that PGI1 synthase is inhibited by 12-HPETE and other hydroperoxides derived from polyunsaturated fatty acids via metabolism by lipooxygenase are particularly significant.5,6,15
Because Ang II stimulates expression of 12-lipoxygenase,1 the administration of lipoxygenase inhibitors baicalein or CDC to rats with Ang II–induced hypertension may promote vascular conversion of PGH2 to PGI2 by minimizing the formation of lipoxygenase-derived fatty acid hydroperoxides.6 Recent studies revealed that superoxide anion production is increased in arterial vessels of rats with Ang II–induced hypertension.24,25 It also is known that prostacyclin synthase is susceptible to inhibition or inactivation by reactive oxygen species26–28 and that flavonoids, such as baicalein, and hydroxycinnamic acid derivatives, such as CDC, possess antioxidant properties.29,30 Accordingly, these lipoxygenase inhibitors also may promote vascular conversion of PGH2 to PGI2 in rats with Ang II–induced hypertension by scavenging oxygen radicals that are damaging to prostacyclin synthase.

In summary, we found that baicalein and CDC lower blood pressure in rats made hypertensive by long-term Ang II infusion but not in normotensive rats. The antihypertensive effect of these lipoxygenase inhibitors was prevented through pretreatment with indomethacin. The antihypertensive effect of baicalein was partially reversed by the administration of 5,6-dihydro-PGI2 antiserum, which binds PGI2 and blocks its vasodepressor action. Treatment of the hypertensive rats with baicalein also caused selective increases in the rate of conversion of exogenous PGH2 to PGI2 by aortic rings, release of 6-keto-PGF1α from aortic rings, concentration of 6-keto-PGF1α in blood, and renal excretion of 6-keto-PGF1α. These data suggest a contribution of PGI2 to the acute antihypertensive effect of baicalein in rats with Ang II–induced hypertension. A vasodepressor prostanoid also appears to contribute to the antihypertensive effect of CDC in rats with Ang II–induced hypertension. Baicalein and CDC may promote PGI2 formation by interfering with the production of one or more inhibitors of PGI2 synthase, including lipoxygenase-derived fatty acid hydroperoxides and reactive oxygen species.

Acknowledgments

This work was supported by grants 5-PO1-HL-4300 and HL-18579 from the National Institutes of Health. We thank Dr. Lawrence Levine from Brandeis University for donating the 5,6-dihydro-PGI2 antiserum used in these studies. We also thank Chiara Kimmel-Preuss and Jennifer Brown for secretarial assistance.

References

Prostaglandin E_2 Contributes to the Vasodepressor Effect of Baicalein in Hypertensive Rats
Hideki Takizawa, AnnMarie DelliPizzi and Alberto Nasjletti

Hypertension. 1998;31:866-871
doi: 10.1161/01.HYP.31.3.866

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/3/866

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/