Arachidonic Acid Elicits Endothelium-Dependent Release From the Rabbit Aorta of a Constrictor Prostanoid Resembling Prostaglandin Endoperoxides

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This study was designed to investigate the mediator(s) of endothelium-dependent arterial constrictor responses evoked by arachidonic acid in vitro. A segment of descending rabbit thoracic aorta was isolated and perfused (1–2 ml/min) with oxygenated Krebs’ bicarbonate buffer. Changes in the vascular smooth muscle–contracting activity of the aortic effluent were detected by superfusion bioassay using either strips of rabbit aorta or rings of dog saphenous vein, both denuded of endothelium and exposed to indomethacin (10 μM). Arachidonic acid (5–50 μg) injected into the inflow of the perfused aorta caused a dose-related increase in the vascular smooth muscle–contracting activity of the aortic effluent, whereas arachidonic acid added directly into the aortic effluent did not. The arachidonic acid–induced elevation of vascular smooth muscle–contracting activity in the aortic effluent was not apparent when indomethacin (10 μM) was added to the aortic inflow to inhibit cyclooxygenase, when the endothelium of the perfused aorta was removed by rubbing, or when the thromboxane A₂/prostaglandin H₂ receptors of the vascular tissues used for bioassay were blocked with an antagonist (1 μM SQ29548), and was unaffected when an inhibitor of thromboxane synthase (10 μM CGS 13080) was added to the aortic inflow. This effect of arachidonic acid was accompanied by release of prostaglandin H₂ (measured as prostaglandin F₂α after reduction with SnCl₂) in amounts sufficient to elicit contraction of the vascular tissues used for bioassay and was attenuated when a reducing agent (2 mM FeCl₂) that converts prostaglandin H₂ to 12-heptadecatrienoic acid was added to the aortic effluent. Collectively, these observations suggest that arachidonic acid stimulates endothelium-dependent release from the perfused aorta of a prostanoid that contracts vascular smooth muscle via interaction with thromboxane A₂/prostaglandin H₂ receptors. The study also suggests that the prostanoid responsible for the vascular smooth muscle–contracting activity of the aortic effluent is a prostaglandin endoperoxide(s) rather than thromboxane A₂. (Circulation Research 1991;69:396–405)

Removal of the endothelium decreases in varying degrees the constrictor response of isolated blood vessels to hypoxia,1 to rapid stretch,2 and to several substances including acetylcholine, calcium ionophore A23187, norepinephrine, and arachidonic acid.3–5 implying that the vasoconstrictor effects of these stimuli are endothelium dependent. Vasoconstrictor events that are endothelium dependent may be mediated by one or more endothelium-derived constrictor factors including endothelin,6 certain oxygen-derived free radicals,7 and metabolites of arachidonic acid.8,9

That metabolites of arachidonic acid by cyclooxygenase contribute to endothelium-dependent vasoconstrictor responses is suggested by reports that inhibitors of cyclooxygenase attenuate many such responses. For example, cyclooxygenase inhibitors were shown to inhibit constrictor responses to arachidonic acid in rabbit aortas8 and in canine cerebral arteries9 and systemic veins.9 Cyclooxygenase inhibitors were also found to reduce constrictor responses to calcium ionophore A23187, acetylcholine, norepinephrine, and stretch in canine basilar arteries2,3 and responses to acetylcholine in rabbit intrapulmonary arteries10 and the aortas of spontaneously hypertensive rats.11

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The identity of the prostanoids mediating endothelium-dependent vasoconstrictor responses is not well established. Inhibitors of thromboxane (TX) synthase reduce constrictor responses to arachidonic acid, norepinephrine, and calcium ionophore A23187 in canine basilar arteries, and to acetylcholine in rabbit intrapulmonary arteries, suggesting mediation of such responses by TXA₂. Yet, inhibitors of TX synthase do not affect constrictor responses to arachidonic acid in the femoral veins of dogs and to acetylcholine in the aortas of spontaneously hypertensive rats. The prostaglandin (PG) endoperoxides, PGG₂ and PGH₂, the common precursors of PGs and TXA₂, cause contraction of blood vessels via interaction with receptors shared with TXA₂ and, consequently, are also candidates for mediating endothelium-dependent vasoconstriction. Recently, the PG endoperoxides were shown to mediate the vasoconstrictor effect of arachidonic acid in the isolated perfused rat kidney.

This paper describes experiments aimed at characterizing the prostanoid(s) mediating contraction of vascular smooth muscle by arachidonic acid. We studied the effect of arachidonic acid on release from the isolated perfused rabbit aorta of vascular smooth muscle–contracting material and characterized the released material in terms of endothelium dependence, relation to cyclooxygenase and TX synthase activities, and ability to implement contraction of vascular smooth muscle via interaction with TXA₂/PGH₂ receptors.

Materials and Methods

Drugs and Solutions

The following agents were used: arachidonic acid, sodium salt (Nu-Chek Laboratories, Elysian, Minn.); CGS 13080 (imidazo[1,5-a]pyridine-5-hexanoic acid, CIBA-GEIGY Corp., Summit, N.J.); SQ29548 ([1S-[1a,2b(5Z),3b,4a]]-7-[3-[2-[[phenylamino]carbonyl]hydrazino][methyl]-7-oxabicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid, E.R. Squibb & Sons, Inc., Princeton, N.J.); BM 13.177 (4-[2-benzensulfonamidoethyl]phenoxycetic acid, Boehringer-Mannheim, Mannheim, FRG); PGE₂, PGF₂α, PGF₃α, U46619 (15S-hydroxy-11α,9α[epoxymethano]prosta-5Z-dienoic acid), and PGH₂ (BIOMOL Research Laboratories, Inc., Plymouth Meeting, Pa.); 12-heptadecatrienoic acid (12-HHT, Cayman Chemical Co., Inc., Ann Arbor, Mich.); acetylcholine chloride, phenylephrine, FeCl₃, and indomethacin (Sigma Chemical Co., St. Louis, Mo.); and SnCl₂ (Allied Chemical Technologies, Inc., Morristown, N.J.).

Arachidonic acid was dissolved in 50 mM Na₂CO₃, titrated to pH 7.0, aliquoted into 1-ml volumes, and stored at −20°C until usage, at which time the solution was thawed and kept under an N₂ stream. PGH₂ was stored in acetone at −70°C; at the time of the experiments it was diluted serially, first with ethanol to a concentration of 1–10 μg/ml and a second time, just before use, with Krebs' bicarbonate buffer to a final concentration of 0.1–1 μg/ml. The concentration of PGH₂ in the final solution was assayed as PGF₂α after reduction with SnCl₂. Indo- methacin was prepared daily in 50 mM NaHCO₃. Stock solutions of CGS 13080 were prepared in 50 mM Na₂CO₃; U46619 and SQ29548, in a mixture of ethanol and 10 mM Na₂CO₃ (0.86:0.14 [vol/vol]); phenylephrine and acetylcholine, in distilled water; and all other agents, in Krebs' bicarbonate buffer.

The composition of Krebs' bicarbonate buffer was (mM) NaCl 118.5, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.1, NaHCO₃ 25.0, and dextrose 11.0.

Animals

Male New Zealand White rabbits (2–3 kg) were anesthetized by intramuscular injections of 50 mg/kg ketamine HCl (Ketaset, Aveco Co., Inc., Fort Dodge, Iowa) and 8 mg/kg xylazine (Rompun, Mobay Corp., Shawnee, Kan.) and, if necessary, by an intravenous injection of 30 mg/kg sodium pentobarbital. After thoracotomy, a 4–6-cm segment of the descending thoracic aorta was excised, immediately placed in ice-cold Krebs' bicarbonate buffer, and carefully cleared of adherent adipose tissue. The aortas were used in experiments to study 1) the effect of arachidonic acid on release of vascular smooth muscle–contracting material from the perfused aorta, 2) the release of PG endoperoxides from the perfused aorta challenged with arachidonic acid, and 3) the mediator of arachidonic acid–induced contraction of aortic rings. In some experiments, saphenous veins were obtained from mongrel dogs anesthetized with 50 mg/kg i.v. sodium pentobarbital and were used to detect release from the perfused rabbit aorta of vascular smooth muscle–contracting material.

Experiments to Study Arachidonic Acid–Induced Release of Vascular Smooth Muscle–Contracting Material From the Perfused Aorta

A 4–6-cm segment of the descending thoracic aorta was cannulated, suspended in a jacketed heating chamber (37°C) that was closed to maintain humidity (100%), and perfused (1–2 ml/min) by means of a peristaltic pump (Buchler Instrument Inc., Fort Lee, N.J.) with gassed (95% O₂–5% CO₂) Krebs' bicarbonate buffer. Selected experiments confirmed that perfusion of the aorta for up to 2 hours does not result in loss of functional damage of aortic endothelial cells. Examination by light microscopy of four perfused aortas stained by silver nitrate revealed that the endothelium lining was always preserved. Aortic rings obtained from four perfused and eight nonperfused aortas, prepared for measurement of isometric tension and contracted by phenylephrine (10⁻⁶ M), were relaxed to the same extent when exposed to acetylcholine (10⁻⁶ M) (i.e., −67±6% nonperfused versus −67±15% perfused), demonstrating preservation of endothelium-dependent vasodilatory responses in the perfused aortas.

Changes in the vascular smooth muscle–contracting activity of the aortic effluent were detected by superfusion bioassay using either a strip of rabbit...
The experiments were initiated by ascertaining that the vascular tissues used for bioassay were appropriately responsive to U46619 injected as a bolus into the effluent of the perfused aorta. Subsequently, arachidonic acid was injected as a bolus into the inflow or the effluent of the perfused aorta, and the response of the strip of rabbit aorta or ring of canine saphenous vein used for bioassay of contracting activity in the effluent was recorded. The purpose of assaying the vascular smooth muscle–contracting activity of the aortic effluent on two vascular preparations was to obtain estimates of such an activity in independent assay systems that are mutually verifiable. When so indicated, to quantify the arachidonic acid–induced increments of vascular smooth muscle–contracting activity in the aortic effluent, the response of assay tissues to arachidonic acid was bracketed between responses in the same assay tissue to two or more injections into the aortic effluent of a reference standard, at varying doses that elicit log-dose–related submaximal contractile responses that are both larger and smaller than the response elicited by arachidonic acid. Other investigators have used a similar approach to quantify by bioassay the release of TXA₂-like materials from isolated perfused organs. In four experiments, the standard of reference used for bioassay of vascular smooth muscle–contracting activity was U46619, a stable analogue of PGH₃, which specifically activates TXA₂/PGE₂ receptors. In four other experiments, the arachidonic acid–induced elevation of vascular smooth muscle–contracting activity in the aortic effluent was assayed on rings of canine saphenous veins using authentic PGH₂ as the standard of reference. Estimates of vascular smooth muscle–contracting activity in the aortic effluent are expressed as picomole equivalents of the appropriate reference standards, U46619 or PGH₃, per unit luminal surface area of perfused aorta. When indicated, the contracting activity of the aortic effluent, measured and expressed as stated above, was multiplied by the surface area of the perfused aorta to obtain estimates of the vascular smooth muscle–contracting activity released from each perfused aorta.

The effect of arachidonic acid on release of vascular smooth muscle–contracting material from the perfused rabbit aorta was studied in preparations with and without endothelium. The endothelium of the perfused aorta was removed by gentle rubbing around polyethylene tubing; the aorta was then flushed with Krebs' bicarbonate buffer and reattached to the perfusion system. The effect of arachidonic acid on release of vascular smooth muscle–contracting material also was studied in the presence and the absence of indomethacin (10 μM) or CGS 13080 (10 μM), added to the inflow of the perfused aorta to inhibit cyclooxygenase and TX synthase, respectively, as well as in the presence and absence of the vascular effluent of ferrous chloride (2 mM), which instantly reduces PGH₂ to 12-HHT, a substance that does not contract rabbit aortic rings at
concentrations as high as $10^{-5}$ M. The role of TXA$_2$/PGH$_2$ receptors in the implementation of the vascular smooth muscle contraction elicited by material released from the perfused aorta by arachidonic acid was investigated by comparing the responses obtained in the presence and the absence of SQ29548 (1 $\mu$M), added to the effluent of the perfused aorta to block TXA$_2$/PGH$_2$ receptors in the vascular tissues used for bioassay.  

In complementary experiments, to study the biological stability of the vascular smooth muscle–contracting material(s) released from the perfused rabbit aorta in response to arachidonic acid, the aortic effluent (2.5 ml) was collected over a 2.5-minute period extending from minute 2.0 to minute 4.5 after injection of a 50-$\mu$g bolus of arachidonic acid into the aortic inflow. The 2.0-minute interval separating the injection of arachidonic acid from the onset of sample collection corresponds approximately to the interval separating the injection of the fatty acid from the appearance of smooth muscle–contracting activity in the aortic effluent. Immediately after completion of the collection, aliquots (100 $\mu$l) of aortic effluent maintained at 37°C were tested at 4-minute intervals for contracting activity on rings of canine saphenous veins superfused with Krebs’ bicarbonate buffer as described above. For the purpose of comparisons, the effluent of perfused aortas that had not been challenged with arachidonic acid was collected for 2 minutes (2 ml), and authentic PGH$_2$ (20 ng) was added to the sample so collected. Immediately thereafter, aliquots (100 $\mu$l) of the PGH$_2$-containing sample were tested at 4-minute intervals for contracting activity as indicated above.

**Experiments to Study Release of PG Endoperoxides From the Perfused Aorta**

Experiments were conducted to examine whether or not the effluent of descending thoracic aortas perfused as described above contains PG endoperoxides after injection of arachidonic acid into the aortic inflow. Arachidonic acid was injected (5- or 50-$\mu$g bolus) into the inflow of the perfused aorta at two time periods separated by a 30-minute interval. After each injection, 2.0 minutes was allowed to elapse before the aortic effluent was collected for 2.5 consecutive minutes. Randomly, one collection was made into 25 ml ethanol containing SnCl$_2$ (5 mg/ml, pH 2.3), and another collection was made into 25 ml ethanol without SnCl$_2$ (adjusted to pH 2.3 with formic acid). Since SnCl$_2$ reduces PGG$_3$ and PGH$_2$ to PGF$_{2\alpha}$, the occurrence of PG endoperoxides in the effluent of perfused aortas challenged with arachidonic acid should be manifested by increased content of PGF$_{2\alpha}$, in samples of effluent collected into ethanol containing SnCl$_2$. To quantify the various prostanoids occurring in the effluent of the perfused aorta, samples collected as described above were extracted with 3 vol ethyl acetate, the organic phase was evaporated to dryness, and the lipid residue that was dissolved in distilled water acidified to pH 3.0 with formic acid was applied to a column of octadecylsilyl silica (Sep-Pak C$_8$ cartridges, Waters Associates, Milford, Mass.) from which prostanoids were eluted as described previously.  

In samples so purified, the content of 6-keto-PGF$_{1\alpha}$, PGE$_2$, PGF$_{2\alpha}$, and TXB$_2$ were determined by enzyme-based immunoassay, using reagents purchased from Cayman Chemical. The content of PG endoperoxides was calculated by subtracting the estimate of PGF$_{2\alpha}$ measured in samples collected without SnCl$_2$ from the corresponding estimate measured in samples collected with SnCl$_2$. The results are expressed as nanograms of eicosanoids released from perfused aortas over 2.5 minutes.

**Experiments to Study the Mediator of Arachidonic Acid–Induced Contraction of Aortic Rings**

Experiments were conducted to examine the role of TXA$_2$/PGH$_2$ receptors in arachidonic acid–induced contraction of rabbit aortic rings. Rings (2–3 mm in length) of descending thoracic aorta suspended in jacketed organ baths (37°C) filled with 5 ml Krebs’ bicarbonate buffer gassed with 95% O$_2$–5% CO$_2$ were connected to force transducers (model FTO3C, Grass Instrument) coupled to a polygraph (model RPS 7P3, Grass Instrument) to measure changes in isometric force. The rings were allowed to equilibrate for 90 minutes at a resting tension of 2 g, with change of buffer at 15-minute intervals. The functional integrity of the endothelium was assessed in all the experiments by evaluating the ability of acetylcholine (10$^{-5}$ M) to relax aortic rings precontracted by phenylephrine (10$^{-6}$ M).

Cumulative concentration–response curves for arachidonic acid were obtained in aortic rings with intact endothelium and in rings denuded of endothelium by gentle rubbing of the intimal surface with a cotton-tipped swab. In vascular rings with endothelium, the effect of arachidonic acid was also studied in the presence and absence of aspirin (500 $\mu$M) or indomethacin (10 $\mu$M) to inhibit cyclooxygenase, in the presence and absence of CGS 13080 (10 $\mu$M) to inhibit thromboxane synthase, and in the presence and absence of SQ29548 (1 $\mu$M) or BM 13.177 (10 $\mu$M) to block TXA$_2$/PGH$_2$ receptors. When arachidonic acid–induced contraction of aortic smooth muscle was examined in the presence of various enzyme inhibitors, receptor antagonists, or other agents, the modifying agent was added to the organ bath 15 minutes before the addition of agonist. In all experiments, agonist-induced contractions are expressed as the increase in absolute tension above resting tension.

**Statistical Analysis**

Results are expressed as mean$\pm$SEM. Agonist concentration–response curves were analyzed by two-way analysis of variance; if differences were noted, the means were compared by Student’s $t$ test. Data on prostanoid released by arachidonic acid from vascular tissue were analyzed by paired $t$ test. The null hypothesis was rejected at $p<0.05$. 

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**Experiments to Study Release of PG Endoperoxides From the Perfused Aorta**

Experiments were conducted to examine whether or not the effluent of descending thoracic aortas perfused as described above contains PG endoperoxides after injection of arachidonic acid into the aortic inflow. Arachidonic acid was injected (5- or 50-$\mu$g bolus) into the inflow of the perfused aorta at two time periods separated by a 30-minute interval. After each injection, 2.0 minutes was allowed to elapse before the aortic effluent was collected for 2.5 consecutive minutes. Randomly, one collection was made into 25 ml ethanol containing SnCl$_2$ (5 mg/ml, pH 2.3), and another collection was made into 25 ml ethanol without SnCl$_2$ (adjusted to pH 2.3 with formic acid). Since SnCl$_2$ reduces PGG$_3$ and PGH$_2$ to PGF$_{2\alpha}$, the occurrence of PG endoperoxides in the effluent of perfused aortas challenged with arachidonic acid should be manifested by increased content of PGF$_{2\alpha}$, in samples of effluent collected into ethanol containing SnCl$_2$. To quantify the various prostanoids occurring in the effluent of the perfused aorta, samples collected as described above were extracted with 3 vol ethyl acetate, the organic phase was evaporated to dryness, and the lipid residue that was dissolved in distilled water acidified to pH 3.0 with formic acid was applied to a column of octadecylsilyl silica (Sep-Pak C$_8$ cartridges, Waters Associates, Milford, Mass.) from which prostanoids were eluted as described previously. 

In samples so purified, the content of 6-keto-PGF$_{1\alpha}$, PGE$_2$, PGF$_{2\alpha}$, and TXB$_2$ were determined by enzyme-based immunoassay, using reagents purchased from Cayman Chemical. The content of PG endoperoxides was calculated by subtracting the estimate of PGF$_{2\alpha}$ measured in samples collected without SnCl$_2$ from the corresponding estimate measured in samples collected with SnCl$_2$. The results are expressed as nanograms of eicosanoids released from perfused aortas over 2.5 minutes.
FIGURE 2. Polygraphic tracings of isotonic responses of helical strips of denuded rabbit thoracic aortas superfused with effluent derived from descending rabbit thoracic aortas perfused with Krebs’ bicarbonate buffer (see “Materials and Methods” for details). Arachidonic acid (AA) and U46619 (a stable analogue of prostaglandin H2 (PGH2)) were injected as a bolus into the inflow of the perfused aorta (through the aorta [TA]) or directly over the assay strip (OAS) into the aortic effluent. AA-induced changes of vascular smooth muscle–contracting activity in the aortic effluent are shown before and after deendothelialization of the perfused vessel (panel A), infusion of indomethacin (INDO, panel B) or thromboxane synthase inhibitor CGS 13080 (panel C) into the aortic inflow (TA), and infusion of receptor antagonist SQ29548 (panel D) or FeCl3 (panel E) into the aortic effluent (OAS).

Results
Effect of Arachidonic Acid on Release of Vascular Smooth Muscle–Contracting Material(s) From the Perfused Aorta

Figures 2 and 3 show representative recordings of responses of strips of rabbit aorta and responses of rings of canine saphenous veins (deendothelialized and continuously superfused with the effluent from rabbit aortas perfused with Krebs’ bicarbonate buffer), respectively, to arachidonic acid injected into the inflow or the effluent of the perfused aortas. After 2.0–2.5 minutes, arachidonic acid injected into the aortic inflow elicited contraction of the strips of rabbit aorta (Figures 2A–2E) and rings of canine saphenous vein (Figure 3A–3D), whereas arachidonic acid injected into the aortic effluent had little or no effect (Figures 2A, 2C, 3A, 3B, and 3D). Hence, the contraction of arterial and venous smooth muscle elicited by arachidonic acid added to the aortic inflow is attributable to release of a contracting material(s) rather than to direct stimulation of vascular smooth muscle.

Mechanical rubbing of the intimal surface of the perfused aorta to remove the endothelium, or addition to the aortic inflow of indomethacin (10 μM) to
inhibit cyclooxygenase, completely prevented the arachidonic acid–induced release of vascular smooth muscle–contracting material(s) (Figures 2A, 2B, 3A, and 3B). The effect of arachidonic acid on increasing the vascular smooth muscle–contracting activity of the aortic effluent was not affected by CGS 13080 (10 μM) added to the inflow of the perfused aorta to inhibit TX synthase (Figures 2C and 3C), was abolished by SQ29548 (1 μM) added to the aortic effluent to block the TXA2/PGH2 receptors of the strips of aorta and rings of saphenous vein used for bioassay (Figures 2D and 3D), and was greatly attenuated by FeCl3 (2 mM) added to the aortic effluent to reduce the PG endoperoxides to 12-HHT (Figure 2E). A synthetic agonist for TXA2/PGH2 receptors, U46619, also elicited contraction of the aortic strips used for bioassay when injected into the effluent of the perfused aorta; this effect of U46619 was blocked by addition to the aortic effluent of SQ29548 but not of FeCl3 (Figures 2D and 2E). SQ29548 also blocked the contraction of canine saphenous vein elicited by addition to the aortic effluent of U46619 or authentic PGH2 (Figure 3D).

As shown in Figure 4A, aortic effluent collected over a 2.5-minute period, from minute 2.0 to minute 4.5, after injection of a 50-μg bolus of arachidonic acid into a perfused aorta, demonstrated vascular smooth muscle–contracting activity when 100-μl aliquots were bioassayed on rings of canine saphenous vein superfused with Krebs’ bicarbonate buffer. Contracting activity was maximal in aliquots of aortic effluent assayed immediately after completion of sample collection (0 minutes) but declined greatly in aliquots taken from samples of effluent kept at 37°C for 4–8 minutes, suggesting that the material(s) responsible for the musculotropism of the aortic effluent is labile. A similar loss of vascular smooth muscle–contracting activity as a function of time at 37°C was noted in samples obtained by mixing authentic PGH2 and the effluent of a perfused aorta not challenged with arachidonic acid (Figure 4B). Before mixing with PGH2, vascular smooth muscle–contracting activity was not detected in the effluent of perfused aortas not challenged with arachidonic acid (data not shown).

Shown in Figure 5, release of vascular smooth muscle–contracting material from the perfused rabbit aorta was quantified independently by bioassay on superfused strips of rabbit aorta and rings of canine saphenous vein using U46619 as the standard of reference. Both bioassay tissues yielded similar estimates of contracting material release, which was found to increase as a function of the amount of arachidonic acid injected. Release of vascular smooth muscle–contracting material from the perfused rabbit aorta also was quantified by bioassay on superfused rings of canine saphenous vein using authentic PGH2 as the standard of reference. In four experiments, arachidonic acid injected as a 5- and 50-μg bolus into the perfused rabbit aorta elicited release of 0.14±0.06 and 1.30±0.17 pmol PGH2 equivalents/cm² luminal surface area, respectively, which corresponds to 0.33±0.14 and 2.82±0.37 ng PGH2 equivalents released per aorta.

**Release of Prostanoids From the Perfused Aorta**

Figure 6 depicts measurements of prostanoids released from perfused rabbit aortas over a 2.5-minute period, from minute 2.0 to minute 4.5, after injections of 5- and 50-μg bolus doses of arachidonic acid. Aortic effluents, collected in both the presence and absence of SnCl2, which is known to reduce PG endoperoxides to PGF2α contained, in decreasing order, PGF2α (measured as 6-keto-PGF1α), PGE2,
PGF2α and TXA2 (measured as TXB2). Estimates of PGI2, PGE2, and TXA2 in samples of aortic effluent collected in the presence of SnCl2 did not differ significantly from corresponding estimates in samples collected without the reducing agent. However, after injection of 5 μg arachidonic acid, four of five samples of aortic effluent collected in the presence of SnCl2 had more \((p<0.08)\) PGF2α than samples collected without SnCl2. After injection of 50 μg arachidonic acid, seven of eight samples of aortic effluent collected in the presence of SnCl2 also had more \((p<0.01)\) PGF2α than samples collected without SnCl2. This suggests occurrence of PG endoperoxides in the effluent of perfused aortas challenged with arachidonic acid. The calculated release of PG endoperoxides from perfused aortas challenged with 5 and 50 μg arachidonic acid was 0.35±0.26 and 1.96±0.26 ng/aorta, respectively.

**Contraction of Aortic Rings by Arachidonic Acid**

Rings of descending rabbit aorta, suspended in an organ bath filled with Krebs’ bicarbonate buffer, developed concentration-dependent increases in tension when challenged with arachidonic acid (Figure 7). This effect of arachidonic acid was not expressed \((p<0.01)\) in aortic rings denuded of endothelium or in rings bathed in media containing either aspirin \((500 \mu M)\) or indomethacin \((10 \mu M)\) \((p<0.001)\) to inhibit cyclooxygenase (Figure 7). The arachidonic acid–induced contraction of rabbit aortic rings was not affected by addition to the bathing media of CGS 13080 \((10 \mu M)\) to inhibit TX synthase but was greatly attenuated by the TXA2/PGF2α antagonists SQ29548 \((1 \mu M)\) \((p<0.001)\) and BM 13.177 \((10 \mu M)\) \((p<0.001)\) (Figure 7).

**Discussion**

The central finding of this study is that arachidonic acid elicits release from the isolated perfused rabbit aorta of material(s) that causes contraction of superfused rings of canine saphenous veins and strips of rabbit aorta. Several complementary findings offer insights into the nature of this effect of arachidonic acid. First, the fatty acid does not release vascular smooth muscle–contracting material(s) from perfused aortas that either are denuded of endothelium or are exposed to buffer containing indomethacin to inhibit cyclooxygenase. Second, the vasoactive material released from the perfused aorta by arachidonic acid does not cause contraction of vascular preparations superfused with buffer containing SQ29548 to block TXA2/PGF2α receptors. Collectively, these observations suggest that the material(s) released by

**Figure 5.** Bar graph illustrating the effect of arachidonic acid on release of vascular smooth muscle contractile activity from descending rabbit thoracic aortas perfused with Krebs’ bicarbonate buffer. Arachidonic acid was injected as a bolus into the aortic inflow, and the contractile activity of the aortic effluent was bioassayed using U46619 (a stable analogue of prostaglandin H2) as standard on either superfused strips of denuded rabbit aorta (RA) or superfused rings of denuded dog saphenous vein (DSV). The values illustrated are the mean±SEM of four experiments in four rabbits.

**Figure 6.** Values of immunoreactive prostanoids released from descending rabbit thoracic aortas perfused with Krebs’ bicarbonate buffer after bolus injections of 5 and 50 μg arachidonic acid. 6-keto-PGF1α, 6-ketoprostaglandin F1α, PGE2, prostaglandin E2; TXB2, thromboxane B2; PGF2α, prostaglandin F2α. Prostanoids were measured in samples collected with and without SnCl2 (see “Materials and Methods” for details). The values illustrated are the mean±SEM of five to eight experiments. *p<0.01 compared with samples collected without SnCl2.
arachidonic acid is a prostanoid(s) that arises via endothelium-dependent mechanisms and contracts vascular smooth muscle via interaction with TXA2/PGH2 receptors.

The production of prostanoids by the rabbit aorta and other large arterial vessels is largely endothelium dependent, because cyclooxygenase activity is more abundant in endothelial cells than in smooth muscle cells. As reported by others, we found that the effluent of perfused aortas challenged with arachidonic acid contains PGF2α along with decreasing amounts of PGE2, PGF2α, and TXA2. That samples of aortic perfusate collected with SnCl2 had more PGF2α than samples collected without SnCl2 is evidence that arachidonic acid also releases from the perfused aorta PG endoperoxides, the immediate precursors of TXA2 and the various PGs. Conceivably, one or more of the prostanoids released from the perfused aorta by arachidonic acid may contribute to the accompanying increase of vascular smooth muscle-contracting activity in the aortic effluent.

PGE2, PGF2α, and PGF1α are known to contract arterial smooth muscle in vitro. According to previous reports, the PGF1-induced contraction of isolated rat and rabbit aorta is endothelium dependent and is mediated by a constrictor prostanoid. In our study, the approximate amount of PGF1 (31.4 ng/aorta), PGE2 (5.5 ng/aorta), or PGF2α (1.6 ng/aorta) released from perfused aortas after a challenge with 50 μg arachidonic acid is below that required by each prostanoid to elicit contraction of the superfused vascular tissues used for bioassay of vasoconstrictor activity in the aortic perfusate (see Figure 1). Hence, it is unlikely that either PG1α, PGE2, or PGF2α contributes significantly to the vascular smooth muscle-contracting activity that is released from the perfused aorta by arachidonic acid.

The possibility that TXA2 contributes to the vascular smooth muscle-contracting activity released by arachidonic acid from the perfused aorta is disputable, because in the present study such a release was unaffected by the TX synthase inhibitor CGS 13080. However, it can be argued that reductions of TXA2 release after inhibition of TX synthase, if accompanied by attendant elevations of PG endoperoxide release, need not necessarily result in diminished vasoconstrictor activity in the aortic effluent. Relevant to this point, it is known that PG endoperoxides and TXA2 share receptors that can be blocked by SQ29548 and that, when activated, result in contraction of vascular smooth muscle.

According to this study performed rabbit aortas, there is general agreement between estimates of 5- and 50-μg arachidonic acid-induced release of PG endoperoxides (0.35 and 1.96 ng/aorta, respectively), measured as PGF2α, and estimates of 5- and 50-μg arachidonic acid–induced release of canine saphenous vein–contracting material determined by bioassay using PGH2 as a standard (0.33 and 2.82 ng/aorta, respectively). The notion that PG endoperoxides contribute to the vascular smooth muscle-contracting activity released by arachidonic acid from the perfused aorta is additionally supported by the finding that the expression of such contracting activity is reduced by the addition of FeCl3 to the aortic effluent superfusing a strip of rabbit aorta used for assay of vasoactivity. FeCl3 is presumed to diminish the concentration of PG endoperoxides in the aortic effluent, as it promotes their reduction to 12-HHT, a substance that is without aortic muscle-contracting activity. According to a recent report, the endothelium-dependent contraction of aortic smooth muscle caused by 20-hydroxyecosatetraenoic acid also appears to be mediated by the corresponding PG endoperoxides that are generated by cyclooxygenase.

From the preceding discussion, PG endoperoxides emerge as the most likely constituents of the vascular smooth muscle-contracting material that is released in an endothelium-dependent manner from the perfused rabbit aorta in response to a challenge with arachidonic acid. PG endoperoxides also may contribute to the mechanism of arachidonic acid–induced contraction of rings of rabbit aorta. This effect of arachidonic acid exhibits dependence on...
both the endothelium and cyclooxygenase activity\(^8\) and, as demonstrated by the present study, is attenuated by blockers of \(\text{TXA}_2/\text{PGH}_2\) receptors but not by an inhibitor of TX synthase. Blockers of \(\text{TXA}_2/\text{PGH}_2\) receptors also were reported to attenuate the expression of acetylcholine-induced contraction of aortic rings from spontaneously hypertensive rats,\(^{29}\) to inhibit the vasoconstrictor effect of arachidonic acid in the isolated perfused rat kidney,\(^{14}\) and to lower blood pressure and produce renal vasodilation in rats with established angiotensin II–salt–induced hypertension.\(^{30, 31}\) In none of these experimental models could the response to blockers of \(\text{TXA}_2/\text{PGH}_2\) receptors be mimicked by inhibitors of TX synthase.\(^{14, 29–31}\) This raises the possibility that the reported effects of the \(\text{TXA}_2/\text{PGH}_2\) receptor blockers reflect interference with the vasoconstrictor actions of a prostanooid other than \(\text{TXA}_2\). Whether or not PG endoperoxides are such prostanooids is yet to be established.

In summary, the present study demonstrates that arachidonic acid elicits endothelium-dependent release from the isolated perfused rabbit aorta of a prostanooid(s) that interacts with \(\text{TXA}_2/\text{PGH}_2\) receptors to cause contraction of vascular smooth muscle. The study also suggests that a PG endoperoxide that contracts vascular smooth muscle and is released from the perfused aorta by arachidonic acid can be such a prostanooid.

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