Methacholine-Induced Contraction of Rabbit Pulmonary Artery: Role of Platelet-Endothelial Transcellular Thromboxane Synthesis

Sandra L. Pfister, David D. Deinhart, William B. Campbell

Abstract—Arachidonic acid- and methacholine-induced contractions of rabbit pulmonary arteries are mediated by thromboxane (TX) A_2. Although removal of the endothelium abolishes the contractions, endothelial cells isolated from pulmonary arteries do not synthesize TXA_2. Further studies described here showed that the expression of TX synthase was evident in platelets and intact pulmonary artery but not in endothelial cells. These studies examined the role of platelet TXA_2 production in the vasoconstrictor response to methacholine. Endothelial cells were incubated with platelets in the presence or absence of methacholine. Methacholine caused an increase in TXB_2 production. Pretreatment of endothelial cells with aspirin (100 μmol/L) before the addition of platelets did not impair the ability of methacholine to increase TXB_2 synthesis. Conversely, if platelets were pretreated with aspirin, methacholine failed to stimulate TXB_2. Using endothelial cells with their cellular lipids labeled with [3H]arachidonic acid, methacholine did not stimulate the production of [3H]TXB_2. When the endothelial cells were incubated with methacholine and control platelets, [3H]TXB_2 was detected. If aspirin-treated platelets were incubated with endothelial cells, methacholine did not increase the production of [3H]TXB_2. However, pretreatment of the endothelial cells with aspirin did not affect the ability of methacholine to induce [3H]TXB_2 release. This suggests that methacholine stimulated the endothelial cell to release arachidonic acid, which was transferred to the platelets and metabolized to TXA_2. To test whether this cell-cell interaction is necessary for methacholine-induced contractions, rabbits were administered aspirin (20 mg/kg) for 2 days. On day 4, methacholine-induced contractions of pulmonary arteries were depressed in aspirin-treated compared with control subjects. Control arteries synthesized 6-keto-prostaglandin F_1α and TXB_2. Aspirin treatment inhibited both pulmonary artery and platelet TXB_2 production but had no effect on vessel 6-keto-prostaglandin F_1α. These studies implicate platelets as a vascular source of TXA_2 and indicate that both endothelial cells and platelets may be required for methacholine-induced TXA_2 synthesis and vasoconstriction (Hypertension. 1998;31[part2]:206-212.)

Key Words: thromboxane A_2 • cyclooxygenase • platelets • arachidonic acid • endothelial cells • endothelium-derived contracting factor

In recent years, the importance of various factors synthesized and released from the blood vessel endothelium that contribute to the regulation of vascular tone has become apparent. In pulmonary vessels, we have identified an endothelium-derived contracting factor as thromboxane (TX) A_2. The control of pulmonary vascular resistance involves the interaction of various vasoconstrictors, such as TXA_2, and vasodilators, such as prostacyclin. Because both prostacyclin and TXA_2 are cyclooxygenase metabolites of arachidonic acid, it is postulated that a balance in these two compounds contributes to the regulation of vascular tone. Abnormalities in the balance of these factors may then have a role in certain pathological states. For example, an increased synthesis of TXA_2 is associated with pulmonary disease, and TXA_2 has been shown to be involved in pulmonary vasoconstriction observed in a number of animal models of pulmonary hypertension.

The major source of TXA_2 is the platelet, polymorphonuclear leukocyte (PMN), and monocyte. Although there are numerous reports of TXA_2 production by blood vessels, there is still controversy as to whether endothelial cells synthesize TXA_2. Several researchers have reported that cultured endothelial cells produce not only prostacyclin but also TXA_2. In contrast, we and others have failed to detect TXB_2 synthesis by endothelial cells. Cultured rabbit pulmonary arterial endothelial cells synthesized 6-keto-prostaglandin (PG) F_1α, the stable metabolite of prostacyclin, but not TXB_2. Immunohistochemical studies indicated the presence of cyclooxygenase, but not TX synthase, in pulmonary artery endothelial cells. Campbell and coworkers reported that primary cultures of umbilical endothelial cells produced TXB_2. However, when the cells were passaged, TXB_2 production was lost. The synthesis of TXB_2 in the primary cultures was...
associated with adherent platelets. With passage, adherent platelets were lost, as was TXB₂ synthesis. Because it is important to identify the cellular source of TXA₂ in pulmonary vessels to assess its role in pulmonary function, the present study was designed to test the hypothesis that TXA₂ synthesis by intact pulmonary arteries requires an interaction between the endothelial cells and adherent platelets. Additional experiments are described that use aspirin-treated rabbits to study the role of the platelet in the vasoconstrictor response to arachidonic acid and methacholine in the intact pulmonary artery.

**Vascular Reactivity**

Two-month-old New Zealand White rabbits were treated with aspirin (3, 10, or 20 mg/kg PO) or its vehicle on days 1 and 2. On day 4, the pulmonary vessels and platelets were isolated as described above. Stips of artery (30 mg, wet weight) or isolated platelets (500 × 10⁶ to 10⁷) obtained from the control and aspirin-treated rabbits were placed in HEPES buffer and incubated at 37°C for 15 minutes with [³H]arachidonic acid (0.05 μCi, 10⁻⁷ mol/L) and the calcium ionophore A23187 (20 μmol/L). The incubation buffer was collected and analyzed using RP-HPLC. To quantitate 6-keto-PGF₁α and TXB₂ production, vessels (3 mg, wet weight) from control and aspirin-treated rabbits were incubated at 37°C in HEPES buffer containing vehicle or arachidonic acid (10⁻⁷ mol/L) for 15 minutes. The synthesis of 6-keto-PGF₁α and TXB₂ was measured by RIA. Additionally, rings (2 to 3 mm) of pulmonary arteries were obtained and suspended in 15 mL organ baths containing Krebs-bicarbonate buffer at 37°C and continuously aerated with 95% O₂/5% CO₂. Isometric tension was measured with Grass force-displacement transducers and recorded with a Grass polygraph (model 7D). Resting tension was adjusted to its length tension maximum of 1 g. The vessels were allowed to equilibrate for 1 hour. Contraction was produced by increasing the KCl concentration of the bath to 40 mmol/L. KCl-induced contractions were repeated until maximal reproducible responses were obtained. Concentration-response curves were obtained by the cumulative addition of either arachidonic acid (10⁻⁸ to 10⁻⁴ mol/L), methacholine (10⁻⁶ to 10⁻⁴ mol/L), or U46619 (10⁻⁵ to 10⁻⁳ mol/L). Because KCl contracture responses were stable throughout the experiment, results were expressed as a percentage of the KCl contraction. Methacholine was dissolved in distilled water, and a volume of 0.05 mL was added to the tissue baths. Arachidonic acid and U46619 were dissolved in ethanol and given in a volume that yielded a final ethanol concentration of the bath of <0.07%.

**Polyacrylamide Gel Electrophoresis and Western Blotting**

Rabbit pulmonary arteries, rabbit platelets, and rabbit pulmonary endothelial cells were obtained as described above. Platelets, endothelial cells, and pulmonary artery lysates were prepared by homogenizing samples in a buffer containing 20 mmol/L HEPES, 255 mmol/L sucrose, 1 mmol/L EDTA, and 100 μmol/L phenylmethylsulfonyl fluoride, pH 7.4. The protein lysates were analyzed by SDS-PAGE by the method of Laemmli using a 4% acrylamide stacking gel and a 10% acrylamide resolving gel. The protein concentration of the pulmonary artery lysates, pulmonary endothelial cell lysates, and platelet lysates was 10 μg. Platelet lysates were also analyzed at a protein concentration of 25 μg. Human platelet microsomes (Biornol Research Laboratories, Inc., Plymouth Meeting, PA) enriched in TX synthase (4 μg) were included as a positive control. The purity of the human microsomal preparation was approximately 47%. The proteins were electrophoretically transferred to nitrocellulose, and the nitrocellulose membrane was blocked for 4 hours at 4°C with 2% nonfat dry milk in 1×-buffered saline (20 mmol/L TRIZMA hydrochloride, 500 mmol/L NaCl, pH 7.5) with Tween-20 (TTBS) before incubation with a polyclonal TX synthase antibody. This polyclonal antibody was made in our laboratory against a unique peptide sequence of TX synthase (NH₂-Cys-Ser-Ile-Ser-Asp-Ala-Asp-Gly-Asp-Glu-Asn-Gly-Lys-Val-COOH). The peptide sequence was synthesized by the Protein and Nucleic Acid Analytical Facility located at the Medical College of Wisconsin.
peptide was conjugated covalently to keyhole limpet hemocyanin and injected with adjuvant into rabbits. Sera from the rabbits was screened for antibody production using an enzyme-linked immunosorbent assay. Preliminary results indicated that rabbits produced an antibody that selectively recognized rabbit platelet TX synthase. The primary antibody was used at a dilution of 1:1000 for 1 hour at 4°C. After washing, the blot was incubated for 20 minutes with the secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG antibody) at a dilution of 1:2000. After again washing with TBS, the blot was incubated for 1 minute with DuPont Renaissance Chemiluminescent reagents. The membrane was subsequently exposed to Kodak Biomax MR imaging film and developed. Pretreated protein markers were used for molecular mass determination.

Statistics

Data are expressed as the mean ± SEM. Statistical analysis of the data was performed with an analysis of variance to determine differences within the groups followed by Dunnet's modification of the t test to determine differences between groups. A value of P < .05 was considered statistically significant.

Materials

[14C]Arachidonic acid was obtained from DuPont NEN (Boston, MA); [1H]6-keto-PGF1α and [1H]TXB2 were from Amersham (Arlington Heights, IL); arachidonic acid was from Nu-Chem Prep, Inc. (Elyria, MND); β-methacholine, A23187, and aspirin were from Sigma; U46619, 6-keto-PGF1α, and TXB2 were from Cayman Chemical Company (Ann Arbor, MI). Thromboxane synthase was from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA). All cell culture reagents were purchased from GIBCO (Grand Island, NY). Flasks used in cell culture were from Corning (Corning, NY). All other chemicals were of reagent grade.

Results

Using a specific polyclonal anti-TX synthase antibody, Western blot analysis showed the presence of immunoreactive bands in lysates prepared from rabbit platelets and rabbit pulmonary arteries (Fig 1). These bands corresponded to the 60-kDa TX synthase protein. Human platelet microsomes enriched in TX synthase were included as a positive control. In pulmonary endothelial cells, the presence of TX synthase was not detected. This experiment was repeated 3 times, and similar results were observed.

We determined whether platelets synthesized TXB2 in response to methacholine. When platelets were incubated with varying concentrations of methacholine, we failed to detect any stimulation of TXB2 synthesis by RIA (data not shown). In contrast, arachidonic acid caused a concentration-related increase in TXB2 synthesis by platelets (data not shown). To determine whether the production of TXB2 by intact pulmonary artery required an interaction between endothelial cells and adherent platelets, endothelial cells and platelets were incubated as described under Methods. As shown previously, endothelial cells alone did not produce TXB2 under either basal (Table 1) or methacholine-stimulated conditions.1 In the presence of endothelial cells and platelets, TXB2 was enhanced approximately 2-fold by the addition of methacholine (Table 1). Because aspirin irreversibly inactivates cyclooxygenase, it was used as a tool to investigate the interaction between these cells. Results showed that when endothelial cells were coincubated with aspirin-treated platelets, methacholine failed to induce TXB2 synthesis (Table 1). In contrast, when aspirin-treated endothelial cells were coincubated with platelets, methacholine-induced TXB2 production was not impaired (Table 1). The production of 6-keto-PGF1α was measured in the coincubation studies (Table 1). The addition of platelets to endothelial cells did not alter the production of 6-keto-PGF1α. If the endothelial cells were pretreated with aspirin, 6-keto-PGF1α production decreased (data not shown). In the presence of normal platelets, aspirin-treated endothelial cell production of 6-keto-PGF1α was still attenuated (Table 1).

These studies were confirmed with [1H]arachidonic acid and the results shown in Fig 2. In radiolabeled endothelial cells incubated with methacholine (10−4 mol/L), there was no production of [1H]TXB2 (Fig 2A). When the endothelial cells were incubated with methacholine in the presence of control platelets, [1H]TXB2 was a major synthetic product (Fig 2B). If aspirin-treated platelets were added to normal endothelial cells, methacholine failed to stimulate the production of [1H]TXB2 (Fig 2C). In contrast, aspirin pretreatment of endothelial cells blocked the production of the cyclooxygenase products 6-ke-

### Table 1. 6-Keto-PGF1α and TXB2 Production in Rabbit Pulmonary Artery Endothelial Cells Coincubated With Rabbit Platelets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6-Keto-PGF1α (pg/mL)</th>
<th>TXB2 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cells + platelets</td>
<td>184 ± 10</td>
<td>172 ± 10</td>
</tr>
<tr>
<td>Endothelial cells + platelets + MECH</td>
<td>254 ± 21</td>
<td>340 ± 49*</td>
</tr>
<tr>
<td>ASA-endothelial cells + platelets + MECH</td>
<td>64 ± 4*</td>
<td>672 ± 33*</td>
</tr>
<tr>
<td>Endothelial cells + ASA-platelets + MECH</td>
<td>159 ± 11</td>
<td>27 ± 4*</td>
</tr>
<tr>
<td>Endothelial cells alone</td>
<td>141 ± 49</td>
<td>ND</td>
</tr>
</tbody>
</table>

Endothelial cells were grown in multwell plates and incubated with rabbit platelets as described under Methods. In some experiments, methacholine (MECH) (10−4 mol/L) was included. Production of 6-keto-PGF1α and TXB2 were measured in the incubation media by radioimmunoassay. Data points are the mean ± SEM for n = 5. ASA (ASA), endothelial cells, or platelets were pretreated with 100 μmol/L ASA before the coincubation studies. ND, not detected.

*P < .05, MECH-stimulated vs control.
Figure 2. Effect of methacholine (10⁻⁴ mol/L) on [³H]arachidonic acid metabolism in prelabeled rabbit endothelial cells incubated alone (A) or in the presence of platelets (B). In C, radiolabeled endothelial cells were pretreated with aspirin (100 µmol/L) for 30 minutes before incubation with control platelets. In D, radiolabeled endothelial cells were incubated with platelets that were previously pretreated with aspirin. The PG metabolites of [³H]arachidonic acid were separated by RP-HPLC. Migration time of known standard eicosanoids are shown above the chromatograms.

to-PGF₂α and -PGE₂, however, pretreatment of the endothelial cells with aspirin did not affect the ability of methacholine to induce TXB₂ release in the presence of normal platelets (Fig 2D). The production of [³H]6-keto-PGF₁α by endothelial cells was not affected by the addition of platelets (Fig 2B) When the endothelial cells were pretreated with aspirin and incubated with normal platelets, no production of [³H]6-keto-PGF₁α was observed (Fig 2D).

The second series of experiments was designed to use aspirin-treated rabbits to further study the role of platelets in the vasoconstrictor response to arachidonic acid and methacholine in the intact pulmonary artery. Arachidonic acid and methacholine produced concentration-related contractions in the control rabbits (Fig 3). However, in the rabbits treated with the highest dose of aspirin (20 mg/kg), there was a significant attenuation of both arachidonic acid- (maximal contraction 66.1 ± 4.4% versus 15.2 ± 3.0%, control versus aspirin-treated, P < 0.05) and methacholine- (maximal contraction 55.4 ± 5.8% versus 16.3 ± 4.0%, control versus aspirin-treated, P < 0.05) induced contractions (Fig 3). The vasoconstrictor response to the TX-mimetic, U46619 was the same in the control and high-dose aspirin-treated animals (data not shown). To assess cyclooxygenase inhibition, pulmonary arteries and platelets from the control and aspirin-treated rabbits were incubated with [¹²⁵I]arachidonic acid and extracted metabolites analyzed by RP-HPLC. Both control (Fig 4A) and high-dose aspirin-treated (Fig 4D) pulmonary arteries synthesized [¹²⁵I]6-keto-PGF₁α, however, its synthesis was less in the vessels from the aspirin-treated rabbits. When measured by RIA, it also seemed that 6-keto-PGF₁α production was reduced in the aspirin-treated rabbit pulmonary arteries (Table 2). High-dose aspirin treatment inhibited the production of [¹³C]TXB₂ (Fig 4B). By HPLC analysis, TXB₂ production was 45.3 ± 18 cps/mg for the control pulmonary arteries and 23.0 ± 8 cps/mg tissue for the aspirin-treated pulmonary arteries. When measured by RIA, there was an approximate 60% reduction in TXB₂ production in the pulmonary arteries obtained from aspirin-treated rabbits compared to the control arteries.
pared with the control rabbits (Table 2). Likewise, in the platelets from the high-dose aspirin-treated rabbits, [14C]TXB2 production was depressed compared with control rabbits (Fig 4C and 4D).

Because 6-keto-PGF1α production was lower in the high-dose aspirin-treated rabbits compared with control rabbits, additional experiments were performed in which rabbits were treated with lower doses of aspirin. The vascular reactivity responses to methacholine in rabbits administered the low-dose regimen (5 mg/kg, PO) or the medium-dose regimen (10 mg/kg, PO) is shown in Fig 3 (bottom). The lower dose of aspirin did not reduce the contractile response to methacholine compared with the control rabbits, whereas the medium dose produced an approximate 50% reduction in methacholine-induced contractions. The low-dose aspirin treatment did not alter either the pulmonary artery or platelet production of [14C]TXB2 compared with the control rabbits (data not shown). In pulmonary arteries obtained from rabbits treated with the 10 mg/kg dose of aspirin, [14C]6-keto-PGF1α production was similar when compared with the control rabbits (Fig 5), however, the platelet [14C]TXB2 production was decreased in the treated rabbits compared with the control rabbits.

**TABLE 2.** 6-Keto-PGF1α and TXB2 Production in Control and Aspirin-Treated Rabbit Pulmonary Artery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6-Keto PGF1α (pg/mg)</th>
<th>TXB2 (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal AA</td>
<td>Basal AA</td>
</tr>
<tr>
<td>Control</td>
<td>2332±426</td>
<td>3374±474</td>
</tr>
<tr>
<td>Aspirin-treated</td>
<td>1031±231</td>
<td>1427±262</td>
</tr>
</tbody>
</table>

Segments of pulmonary arteries from control or aspirin-treated rabbits were incubated with vehicle or arachidonic acid (AA) (10⁻³ mol/L) for 15 minutes at 37°C. Production of 6-keto-PGF1α and TXB2 were measured in the incubation media by radioimmunoassay. Values are the mean±SEM for n=6 vessel segments.

**Discussion**

Our original study reported that endothelium-dependent contractions are produced by arachidonic acid and methacholine in rabbit intrapulmonary artery. Using a variety of techniques, including vascular reactivity studies with inhibitors, bioassay,
HPLC, RIA, and gas chromatography/mass spectrometry analysis, our previous work conclusively showed that TXA₂ is the mediator of these contractions. However, the question remained as to which cell type(s) in the intact pulmonary artery produce TXA₂. Although there are numerous reports of TXA₂ production by blood vessels, controversy exists as to whether endothelial cells synthesize TXA₂. Some researchers have reported that cultured endothelial cells produce not only prostacyclin but also TXA₂. In contrast, other studies did not detect TXA₂ synthesis by endothelial cells. In our previous study, immunofluorescence of rabbit pulmonary endothelial cells failed to detect the presence of TX synthase, whereas the presence of cyclooxygenase was clearly shown. The present study further supported the absence of TX synthase in cultured rabbit pulmonary artery endothelial cells. Using a specific polyclonal anti-TX synthase antibody, Western blot analysis showed the presence of immunoreactive bands in lysates prepared from rabbit platelets and intact rabbit pulmonary artery. These bands corresponded to the 60-kD TX synthase protein purified from human platelets and co-migrated with a human platelet microsome enriched in TX synthase. However, in lysates prepared from the rabbit pulmonary artery endothelial cells, no immunoreactive band was observed. Our previous experiments also showed that endothelial cells incubated with [³⁵S]arachidonic acid did not produce [³⁵S]TXB₂. When a more sensitive RIA method was used to quantitate TXB₂ production under both basal and stimulated conditions, no detectable TXB₂ was measured. Taken collectively, these studies would be consistent with some cell type other than endothelial cells releasing TXA₂. Because TXA₂ is the major arachidonic acid metabolite in platelets, we tested the hypothesis that TXA₂ synthesis by intact pulmonary arteries requires an interaction between endothelial cells and platelets.

We showed that methacholine-stimulated TXB₂ synthesis in intact pulmonary artery. However, incubation of platelets with methacholine does not stimulate TXB₂ production, and cholinergic receptors have not been reported to exist on platelets. Therefore, a question remains as to how methacholine enhances the release of TXA₂ from the platelet in the intact pulmonary artery. PGH₂ from stimulated platelets may be taken up by endothelial cells and used to produce PGI₂; however, this transfer of PGH₂ does not occur in the reverse direction, i.e., platelets do not take up PGI₂ from endothelial cells for TXA₂ synthesis. It is possible that endothelial cells will, however, transfer arachidonic acid to platelets and promote TXB₂ synthesis. We investigated this interaction in cultured endothelial cells and platelets during methacholine stimulation. Methacholine stimulated TXB₂ synthesis in platelets and endothelial cells but not in platelets alone or in endothelial cells alone, supporting our hypothesis that an interaction between these two cell types is required for methacholine-induced TXA₂ synthesis. Additional experiments used aspirin as a tool to confirm that arachidonic acid and not the cyclooxygenase metabolite PGH₂ was transferred to the platelet and further metabolized to TXA₂. Aspirin irreversibly inactivates cyclooxygenase by acetylating the enzyme. Pretreatment of platelets with aspirin blocks methacholine-induced TXB₂ synthesis when platelets and endothelial cells are cocultured, indicating that platelet cyclooxygenase is required. These data suggest that endothelial cells release arachidonic acid on cholinergic stimulation and that the platelets convert this arachidonic acid to TXB₂.

To further support this conclusion, aspirin-treated endothelial cells and platelets produced TXB₂ in response to methacholine, indicating that endothelial cell cyclooxygenase is not required and suggesting that endothelial cell-derived arachidonic acid is the source of TXB₂. This conclusion is represented schematically in Fig 6.

The last series of experiments used aspirin-treated rabbits to study the role of the platelet in the vasomotor response to arachidonic acid and methacholine in the intact pulmonary artery. It has been documented that aspirin can selectively inhibit platelet cyclooxygenase because of the inability of platelets to regenerate their cyclooxygenase. Thus, aspirin has a longer duration of action in platelets than in endothelial cells. This treatment regimen was designed to selectively inhibit platelet, but not endothelial, cyclooxygenase. In rabbits treated with high and medium doses of aspirin, platelet and pulmonary artery [³⁵S]TXB₂ synthesis was inhibited. Pulmonary artery production of [³⁵S]6-keto-PGF₁α was reduced in the high-dose aspirin-treated rabbits compared with the control rabbits. However, at the lower doses of aspirin, pulmonary artery 6-keto-PGF₁α production was similar between the treated and control rabbits. The vasomotor responses to methacholine and arachidonic acid were reduced in vessels from the medium- and high-dose aspirin-treated rabbits but not in vessels from control rabbits or in rabbits treated with the low-dose aspirin regimen. The response to U46619 was the same in the vessels from control and aspirin-treated animals. Because platelet TXA₂ production is decreased in the aspirin-treated rabbits that exhibited decreased contractile response to methacholine, these results would implicate platelets as the cellular source of TXA₂ production. It is important to note that a recent report by Barry et
Platelet-Derived Thromboxane and Pulmonary Artery Contractions

al has shown that microparticles released from platelets also play a role in the transcellular metabolism of arachidonic acid. Specifically, platelet microparticles were shown to transfer arachidonic acid to the platelets to be used for TXA2 synthesis. Although this mechanism was not explored in the present study, it gives further support for how platelets can influence vascular function.

Although the major source of TXA2 is the platelet, PMN and monocyte also produce TXA2. Therefore, PMNs or monocytes may also be possible sources of TX synthase in the rabbit pulmonary artery. Because this potential mechanism was not examined in the present study, the role of PMNs and monocytes cannot be eliminated.

Therefore, we propose that methacholine-induced contractions of rabbit pulmonary artery are mediated by the release of arachidonic acid from endothelial cells and its transfer to adherent platelets that subsequently synthesize the contracting factor TXA2. TXA2 is released by the platelet and has a direct effect on vascular smooth muscle TXA2 receptors. These studies support the concept of transcellular metabolism of arachidonic acid in the pulmonary vasculature. Because TXA2 is an important mediator of pulmonary hypertension caused by a variety of pathophysiological conditions, the identity of the platelet, and not the endothelium, as a cellular source of TXA2, may provide further insight into the role of platelets in the regulation of pulmonary vascular tone.

Acknowledgments

We thank Joseph James and Donna Kotulock for technical assistance and Gretchen Barg for secretarial assistance. These studies were supported by grants from the National Heart, Lung, Blood Institute (HL-37981 and HL-57895).

References

20. Rebyka GR, Johnson AB, Campbell WB. Cultured bovine coronary artery endothelial cells synthesize HETEs and prostacyclin. Am J Physiol 1988;254:C8–C19

Downloaded from http://hyper.ahajournals.org/ on July 12, 2017
Methacholine-Induced Contraction of Rabbit Pulmonary Artery: Role of Platelet-Endothelial Transcellular Thromboxane Synthesis
Sandra L. Pfister, David D. Deinhart and William B. Campbell

Hypertension. 1998;31:206-212
doi: 10.1161/01.HYP.31.1.206

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/31/1/206

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/