Oxygen Metabolites Stimulate Thromboxane Production and Vasoconstriction in Isolated Saline-perfused Rabbit Lungs

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Abstract. Generation of reactive oxygen metabolites, thromboxane increases, and vasoconstriction have been implicated in the pathogenesis of acute edematous lung injury, such as that seen in patients with the Adult Respiratory Distress Syndrome (ARDS), but their interactions are unknown. We hypothesized that reactive O_2 products would stimulate arachidonic acid metabolism in lungs and that vasoactive products of arachidonate, such as the potent vasoconstrictor thromboxane A_2, might then mediate O_2-metabolite-induced pulmonary vasoconstriction. We found that O_2 metabolites generated by injection of purine plus xanthine oxidase caused increases in mean pulmonary artery perfusion pressures (27±4 mmHg) in isolated perfused lungs. In addition, purine plus xanthine oxidase also caused 30-fold increases in perfusate levels of thromboxane B_2 (the stable metabolite of thromboxane A_2) compared with only twofold increases in 6-keto-PGF_{1a} (the stable metabolite of prostacyclin). Moreover, prior addition of catalase inhibited both vasoconstriction and the thromboxane B_2 production seen in isolated lungs following injection of purine plus xanthine oxidase. Similarly, pretreatment with cyclooxygenase inhibitors, either aspirin or indomethacin, also completely blocked thromboxane generation and markedly attenuated pressor responses usually seen after purine plus xanthine oxidase (increase in mean pulmonary artery perfusion pressures, 4.4±1.5 mmHg). Furthermore, imidazole, a thromboxane synthetase inhibitor, also decreased O_2-metabolite-induced thromboxane generation and vasoconstriction. These results suggested that thromboxane generation might participate in O_2-metabolite-induced vasoconstriction. However, since a significant correlation between thromboxane levels and the degree of vasoconstriction could not be demonstrated, and since addition of superoxide dismutase reduced thromboxane generation but did not affect the intensity of vasoconstriction, it is possible that thromboxane is not the only vasoactive mediator in this model. We conclude that exposing lungs to O_2 metabolites results in thromboxane generation and that thromboxane is a major mediator of oxidant-induced vasoconstriction.

Introduction

Reactive oxygen metabolites from neutrophils and other sources appear to contribute to the development of acute edematous lung injury, such as that seen in the Adult Respiratory Distress Syndrome (ARDS) and other disorders (1–10). Recently, we found that hydrogen peroxide (H_2O_2) or H_2O_2-derived products caused pulmonary vasoconstriction and permeability pulmonary edema in isolated saline-perfused rabbit lungs (11). Since O_2 metabolites can interact with cell membranes (12) and since cell membrane stimulation is frequently associated with liberation of arachidonic acid from membrane phospholipids (13), it appeared that intermediate arachidonate-derived factors might be involved in O_2-metabolite-induced vasoconstriction or pulmonary edema. Based on this possibility, we hypothesized that O_2 metabolites stimulate arachidonate metabolism and that production and release of potent vasoactive arachidonate products, such as thromboxane, might in part mediate oxidant-induced vasoconstriction. This hypothesis was supported by finding that exposure of isolated lungs to O_2 metabolites generated by xanthine oxidase caused generation of large amounts of thromboxane and that addition of catalase, an H_2O_2 scavenger, aspirin or indomethacin, cyclooxygenase inhibitors, or imidazole, a thromboxane synthetase inhibitor, similarly decreased both per-

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fusate thromboxane increases and elevations of mean perfusion pressures in isolated lungs perfused with O_2 metabolites produced by xanthine oxidase.

Methods

Reagents. Xanthine oxidase (grade I, buttermilk), purine (7H-Imidazo [4,5-*d*] pyrimidine), glucose oxidase (type V, Aspergillus niger), β-D (+) glucose, superoxide dismutase (SOD[1], type I, 2,650 U/mg protein), catalase (bovine liver, 2,000 U/mg protein), acetyl salicylic acid (aspirin), indomethacin, imidazole, bovine serum albumin (BSA, Cohn fraction V), bromcresol green (albumin color reagent), and horse-heart ferricytochrome *c* were obtained from Sigma Chemical Co., St. Louis, MO. Papaverine hydrochloride was obtained from Eli Lilly and Co., Indianapolis, IN. In some experiments, catalase was further purified by passage through a Sephadex G-100 column, concentrated and desalted by pressure dialysis (Amicon Ultrafiltration System, Diaflo XM 100 membrane, Amicon Corp., Danvers, MA), and lyophilized.

Isolated perfused lung standard protocol. New Zealand White rabbits (1.8–2.8 kg) were used for all isolated lung preparations. Each rabbit was anesthetized with xylazine (Rompun, Cutter Laboratories, Shawnee, KS; 10 mg/kg intramuscularly) and ketamine (Ketalar, Parke-Davis & Co., Detroit, MI; 25–50 mg/kg intravenously). A tracheostomy was performed and each rabbit was ventilated with 95% room air:5% CO_2 during the operative procedure (Harvard small animal respirator, model 670D, Harvard Apparatus Co., Inc. Minnis, MA; fixed tidal volume, 10–15 cm^3/kg, 25 breaths/min; positive end-expiratory pressure, 2 cm H_2O). A median sternotomy was performed and 500 U heparin (Panheprin, Abbott Laboratories, N. Chicago, IL; 1,000 U/ml) was injected into the right ventricular cavity. Cannulas were then placed in the main pulmonary artery and left ventricular cavity. The perfusate medium was Greenberg-Bohr physiological saline solution (14) containing 3% BSA at 37°C and pH 7.4. The pulmonary circulation was washed with 500 ml of perfusate using a peristaltic pump (perfusate flow rate, 40 cm^3/kg body weight per min, Cole-Parmer Instrument Co., Chicago, IL). This washout procedure reduced subsequent recirculating perfusate neutrophil and platelet counts to <3% of normal rabbit values. Perfusion was then interrupted for 5 min while the lungs and heart were dissected free and suspended in a humid 37°C chamber from a force-displacement transducer (model FT10 G, Grass Instruments Co., Quincy, MA) that allowed continuous measurement of lung preparation weight changes. Perfusion was then restarted at the same fixed flow rate. The final recirculating volume was 300 ml. The level of the venous outflow line was kept ~10 cm below the lung preparation, causing outflow pressure to be ≤0 mmHg. Mean perfusion pressures were monitored with a pressure transducer (type 4-327-0010, Bell & Howell Co., Pasadena, CA) zeroed at the level of the main pulmonary artery cannula. Perfusion pressures initially ranged from 8 to 12 mmHg in all preparations and were followed throughout each experiment (see Results). Each preparation was perfused for 10–20 min prior to experimental manipulations to insure stability. During the base-line period, perfusion pressures and weights in individual preparations varied <3 mmHg and 2 g, respectively. Experimental agents were added to the perfusate reservoir after 10, 20, or 25 min in the following order: O_2 radical scavenger or arachidonic acid metabolism inhibitor, substrate, and enzyme. Perfusion was continued for an additional 30 min (55 min total) or until gross edema necessitated an earlier termination. In selected experiments, papaverine was injected into the perfusate after the addition of purine and xanthine oxidase and was titrated (average final papaverine concentration, 0.1 mg/ml) to maintain nearly constant perfusion pressures. All perfusion pressure data are reported as the maximum pressure increases prior to any lung weight gains.

Measurement of thromboxane B_2 and 6-keto-prostaglandin F_{1a} (PGF_{1a}). Perfusate samples (3 ml) were drawn from the venous outflow line before and after addition of xanthine oxidase. Samples were then immediately mixed with 20 μl of 0.4% aspirin in phosphate-buffered saline, pH 7.0. Subsequently, the samples were centrifuged (500 *g* for 8 min at 4°C) and supernatants were stored at −70°C until assayed. Unextracted samples (0.2 ml) were assayed in duplicate by a double-antibody radioimmunoassay procedure (15). The assay sensitivity for thromboxane B_2 and 6-keto-PGF_{1a} is 0.005–0.010 ng/tube, with a coefficient of variation for repetitive assays of 10% (15).

Measurement of superoxide anion (O_2^-). Aspirin, indomethacin, or imidazole did not interfere with purine plus xanthine oxidase-induced O_2 metabolite production as measured by O_2^--dependent reduction of horse heart ferricytochrome *c* spectrophotometrically (16).

Statistics. Data are reported as mean±SEM except where indicated. Comparisons between purine-xanthine oxidase or β-D glucose-glucose oxidase-exposed lungs and multiple groups were made by a one-way analysis of variance followed by Scheffe's Multiple Comparison Test (17). Probability values < 0.05 were considered significant.

Results

Addition of purine plus xanthine oxidase to perfusates of isolated saline-perfused rabbit lungs caused marked increases in lung perfusion pressures that were abolished by preaddition of catalase (Fig. 1). This observation suggested that H_2O_2 or an H_2O_2-derived product was responsible for vasoconstriction. In contrast, addition of SOD, a scavenger of superoxide anion (O_2^-), did not alter purine plus xanthine oxidase-mediated increases in perfusion pressures, suggesting that O_2^- did not cause these responses (Fig. 1). The contention that H_2O_2, and not O_2^-, initiated these pressure changes was further supported when glucose oxidase, an enzyme that generates H_2O_2 but not O_2^- (6), caused similar increases in lung perfusion pressures that were inhibitable by preaddition of catalase (Fig. 2).

Addition of purine plus xanthine oxidase also caused 30-fold increases in thromboxane B_2 levels (the stable metabolite of thromboxane A_2, Fig. 3) while producing only twofold increases in 6-keto-PGF_{1a} levels (the stable metabolite of prostacyclin, Fig. 4) in perfusates of isolated lungs. Specifically, 15 min after addition of purine plus xanthine oxidase, lung perfusates had thromboxane B_2 and 6-keto-PGF_{1a} levels of 0.43±0.19 and 0.76±1.5 ng/ml (mean±SD) compared with 0.02±0.02 and 0.37±0.10 ng/ml at the start of the experiments. The rate of thromboxane generation was approximately linear during the first 30 min after addition of xanthine oxidase. Addition of catalase or SOD inhibited purine plus xanthine oxidase-mediated rises in thromboxane B_2 levels (Fig. 3). Catalase, but not SOD, inhibited the purine plus xanthine oxidase-induced increases in 6-keto-PGF_{1a} levels (Fig. 4).

Having observed this apparent stimulation of arachidonate metabolism by xanthine oxidase-derived O_2 metabolites, we next examined whether thromboxane generation contributed to O_2-

1. *Abbreviations used in this paper:* PGF_{1a}, prostaglandin F_{1a}; SOD, superoxide dismutase.

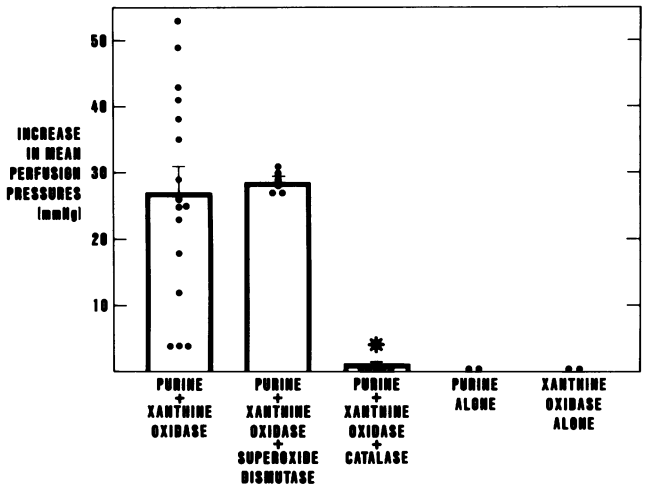

Figure 1. Purine (2 mM) plus xanthine oxidase (0.02 U/ml) caused increases in mean perfusion pressures in isolated lungs. All perfusion pressure data are reported as the maximum pressure increases prior to any lung weight gains. Superoxide dismutase (100 μg/ml, $n = 4$; 10 μg/ml, $n = 1$; 1 μg/ml, $n = 1$) did not alter the xanthine oxidase-induced pressor responses. Catalase (100 μg/ml, $n = 4$) or purified catalase (50 μg/ml, $n = 2$) inhibited the pressor responses. Each symbol represents one experiment. Asterisk indicates significant difference ($P < 0.05$) from purine plus xanthine oxidase group.

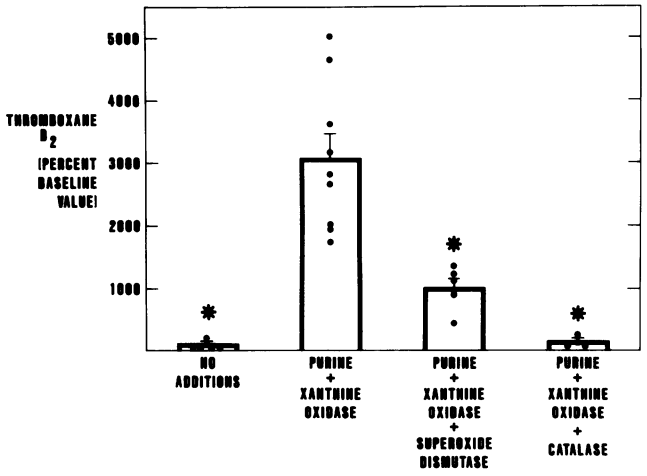

Figure 3. Purine (2 mM) plus xanthine oxidase (0.02 U/ml) caused 30-fold increases in thromboxane B_2 levels in isolated lung perfusates. Thromboxane B_2 is expressed as percent base-line value (thromboxane B_2 value at 15 min after purine plus xanthine oxidase divided by thromboxane B_2 value at 15 min before xanthine oxidase × 100). Superoxide dismutase (100 μg/ml, $n = 4$; 10 μg/ml, $n = 1$; 1 μg/ml, $n = 1$) or catalase (100 μg/ml) inhibited xanthine oxidase-induced increases in thromboxane B_2. Each symbol represents one experiment. Asterisk indicates significant difference ($P < 0.05$) from purine plus xanthine oxidase group.

metabolite-induced vasoconstriction by determining if cyclooxygenase or thromboxane synthetase inhibitors would attenuate thromboxane generation and pressor responses seen after addition of purine plus xanthine oxidase. We found that purine plus xanthine oxidase-induced pressor responses in isolated lungs

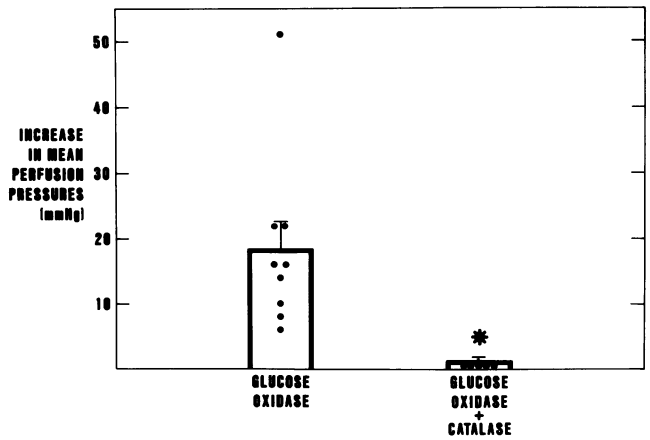

Figure 2. Glucose oxidase (0.04 U/ml) plus β-D glucose (1%) caused increases in mean perfusion pressures in isolated lungs. Catalase (100 μg/ml) inhibited the β-D glucose plus glucose oxidase-induced pressor responses. Each symbol represents one experiment. Asterisk indicates significant difference ($P < 0.05$).

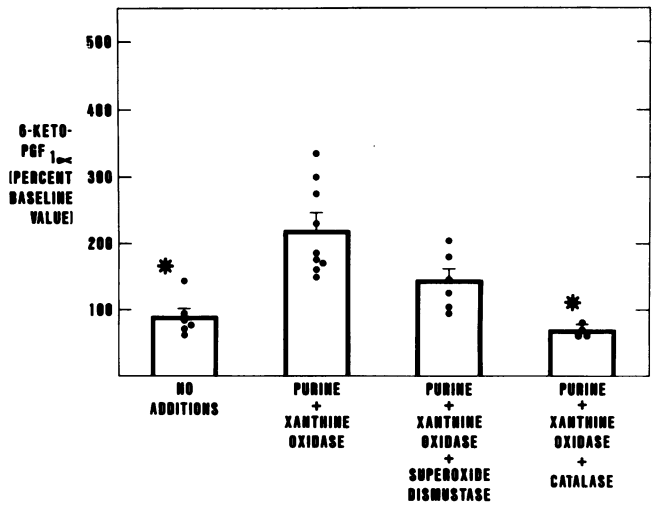

Figure 4. Purine (2 mM) plus xanthine oxidase (0.02 U/ml) caused twofold increases in 6-keto-PGF_{1a} levels in isolated lung perfusates. 6-keto-PGF_{1a} is expressed as percent base-line value (6-keto-PGF_{1a} value at 15 min after purine plus xanthine oxidase divided by 6-keto-PGF_{1a} value at 15 min before xanthine oxidase × 100). Catalase (100 μg/ml), but not superoxide dismutase (100 μg/ml, $n = 4$; 10 μg/ml, $n = 1$; 1 μg/ml, $n = 1$), inhibited xanthine oxidase-induced increases in 6-keto-PGF_{1a}. Each symbol represents one experiment. Asterisk indicates significant difference ($P < 0.05$) from purine plus xanthine oxidase group.

were inhibited by preaddition of either of two cyclooxygenase inhibitors, aspirin or indomethacin (Fig. 5). Addition of aspirin or indomethacin also abolished increases in thromboxane B_2 and 6-keto-PGF_{1a} levels. Furthermore, imidazole, a thromboxane synthetase inhibitor (18), also attenuated purine plus xanthine oxidase-induced pressor responses (Fig. 6). At the higher dose (7.3 mM), imidazole decreased production of thromboxane B_2 but increased 6-keto-PGF_{1a} production (6-keto-PGF_{1a} levels increased 3.7-fold). Thus, concentrations of imidazole required to inhibit thromboxane synthetase in our system did not adversely affect cyclooxygenase activity. Additional control experiments demonstrated that aspirin, indomethacin, and imidazole had no effect on the generation of O_2^- by xanthine oxidase in vitro as evidenced by their noninterference in a standard cytochrome *c* reduction assay. Also, addition of catalase, SOD, imidazole, aspirin, or indomethacin alone did not affect base-line perfusion pressures in any experiment (data not shown).

Finally, we examined if thromboxane generation may have been a consequence of O_2-metabolite-induced vasoconstriction. Injection of the vasodilator, papaverine, attenuated xanthine oxidase-induced pressor responses by 80% (increase in mean perfusion pressures, 4.7±0.6 mmHg, $n = 3$), but did not affect the increase in thromboxane levels (4,016±2,405, percent base line). This finding suggested that exposure to O_2 metabolites caused thromboxane generation independently of vasoconstriction.

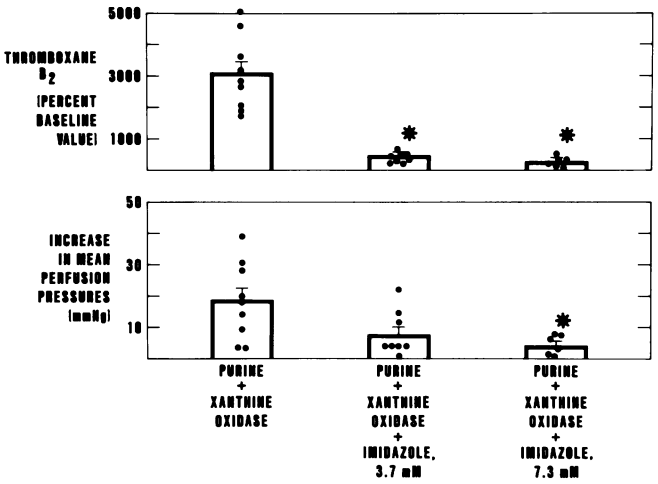

Figure 6. Imidazole attenuated xanthine oxidase-induced increases in thromboxane B_2 levels and increases in mean perfusion pressures. Imidazole was examined for any nonspecific vasodilator activity. Imidazole alone did not affect base-line perfusion pressures. Furthermore, imidazole did not affect potassium chloride-induced vasoconstriction ($n = 3$). Each symbol represents one experiment. Asterisk indicates significant difference ($P < 0.05$) from purine plus xanthine oxidase group.

Discussion

This investigation shows that xanthine oxidase-derived O_2 metabolites can stimulate the generation of thromboxane in isolated saline-perfused rabbit lungs. Furthermore, O_2 metabolite-induced thromboxane generation appears to be an important mechanism responsible for oxidant-induced vasoconstriction.

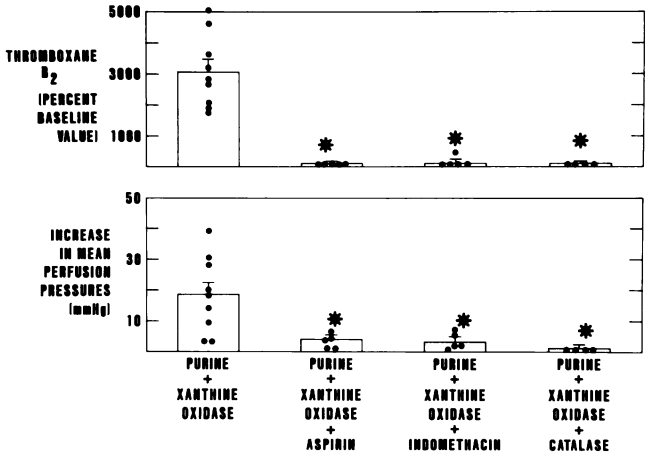

Figure 5. Aspirin (0.8 mM) or indomethacin (30 mM) attenuated xanthine oxidase-induced increases in thromboxane B_2 levels and increases in mean perfusion pressures. Despite complete inhibition of xanthine oxidase-induced increases in thromboxane B_2 levels by aspirin or indomethacin, ~20% of the mean pressor response remained. This residual pressor response was not seen in the presence of catalase. Each symbol represents one experiment. Asterisk indicates significant difference ($P < 0.05$) from purine plus xanthine oxidase group.

Considerable evidence suggests that oxygen metabolites could cause thromboxane generation by interacting at several sites in the pathways of arachidonic acid metabolism (19–24). Our finding that cyclooxygenase inhibitors effectively blocked xanthine oxidase-induced thromboxane generation suggested that xanthine oxidase-derived O_2 metabolites were affecting the initial steps of arachidonate metabolism, including arachidonic acid release from phospholipids or cyclooxygenase enzyme activity. The enormous increase in thromboxane levels in our system indicates that O_2 metabolites most likely caused increased liberation of substrate arachidonic acid from cell membranes in some fashion. Two major possibilities seem to exist. It is known that O_2 metabolites can attack unsaturated bonds of membrane lipids (12), and that this process of lipid peroxidation can become autocatalytic and lead to changes in membrane structure that allow for increased release of membrane-bound arachidonic acid. Indeed, cell membrane alterations often result in release of arachidonic acid or its products (13). Another possibility would be stimulation of phospholipases by O_2 metabolites. The latter is an attractive mechanism which has not been described. O_2 metabolites might also directly stimulate cyclooxygenase. Indeed, various peroxides can accelerate cyclooxygenase activity (25, 26). Although our investigation did not attempt to determine which of these several possibilities was most important for O_2-metabolite-induced thromboxane generation, we consider the direct perturbation of cell membranes by O_2 metabolites to be

the most likely mechanism leading to production of arachidonate metabolites in this system.

Another issue raised by our findings is the cellular site of production of arachidonate metabolites seen in isolated lungs exposed to O_2 metabolites. Since most mammalian cells have the metabolic capability of generating arachidonate products (13, 27), it is probable that resident lung cells, such as endothelial cells or fibroblasts, contribute to the thromboxane and prostacyclin generation. In contrast, it is less probable that circulating blood cells contribute. For example, it is unlikely that platelets are the source of thromboxane in this model. The isolated lung vasculature is thoroughly washed prior to the beginning of each experiment. As a result of this washing procedure, circulating platelets are not detectable in the perfusing saline solution. Furthermore, lung sections prepared for light and electron microscopy reveal very few residual platelets. Another possibility is that neutrophils contribute to thromboxane generation. However, while some neutrophils are apparent in lung histologic sections, the numbers of circulating neutrophils are so markedly decreased in isolated lung perfusates that it seems only remotely possible that they are the major source of thromboxane.

A relationship between O_2-metabolite-induced thromboxane generation and vasoconstriction seems likely. Thromboxane is a well-recognized, potent vasoconstrictor. Moreover, addition of catalase completely inhibited purine-xanthine oxidase-induced thromboxane generation and no vasoconstriction occurred. Furthermore, aspirin, indomethacin, or imidazole inhibited purine-xanthine oxidase-induced vasoconstriction and thromboxane generation without affecting oxygen radical generation. These results suggest that thromboxane was a mediator of oxidant-induced vasoconstriction. However, other findings in our study suggest that thromboxane may not be the only vasoconstrictor in this model. For example, SOD significantly reduces thromboxane generation; yet, a pressor response still occurs. However, as shown in Fig. 3, the amount of thromboxane generated in the presence of SOD is greater than in the isolated lungs with no additions. It may be that thromboxane generation must be almost totally suppressed to prevent O_2-metabolite-induced vasoconstriction. A second concern regarding the potential contribution of mediators other than thromboxane is that a strict correlation does not exist between the amount of thromboxane generated and the intensity of vasoconstriction. This does not mean that thromboxane is not involved but rather suggests that O_2-metabolite-induced vasoconstriction potentially involves more than one vasoactive substance. For instance, even when aspirin or indomethacin totally inhibited the rise in thromboxane levels, a small pressor response remained (4.4±1.5 mmHg, Fig. 5), suggesting that a vasoactive substance in addition to thromboxane was present in the system. A possible explanation for the small pressor response seen in the presence of cyclooxygenase inhibitors would be that arachidonic acid was shunted away from the cyclooxygenase pathway into the lipoxygenase pathway. This effect has been shown to exist in platelets where aspirin decreased cyclooxygenase products while simultaneously increasing lipoxygenase products (28). If this phenomenon occurred in our system, the aforementioned residual pressor response seen in the presence of cyclooxygenase inhibitors may have been caused by a vasoconstricting lipoxygenase product (29). Additional factors that might account for the lack of a strict correlation between thromboxane levels and the degree of vasoconstriction are variabilities in (*a*) the reactivity of pulmonary vascular beds between rabbits, (*b*) the responsiveness of individual rabbits to thromboxane, prostacyclin, and/or other factors, and (*c*) factors that affect the binding of thromboxane to its receptor. These variables cannot be readily measured in our system. To summarize, it is clear that arachidonate metabolites are generated in response to O_2 metabolites in this model. Furthermore, it appears that although thromboxane very likely mediates a major part of O_2-metabolite-induced vasoconstriction, other factors which stimulate vasoconstriction may also be involved.

The finding that low-dose imidazole (3.7 mM, Fig. 6) reduced thromboxane levels without significantly reducing purine-xanthine oxidase-induced vasoconstriction raised the possibility that imidazole was effective at the higher dose (7.3 mM) by acting as a nonspecific vasodilator in addition to a thromboxane synthetase inhibitor. We are not aware of imidazole being reported as a nonspecific vasodilator. As noted in Fig. 6, imidazole had no vasodilator effect either on base-line perfusion pressures or on potassium chloride-induced vasoconstriction. Thus, it may well be that thromboxane generation must be almost totally suppressed by the higher dose of imidazole to prevent O_2-metabolite-induced vasoconstriction. Similarly, we were not able to demonstrate any nonspecific vasodilating effect of catalase. Furthermore, we are not aware that such an effect of catalase has been described.

Thromboxane-dependent pulmonary vasoconstriction has been demonstrated in several models. Infusion of endotoxin into dogs or sheep raises pulmonary vascular resistances (30–34). This effect apparently involves arachidonate products since the resulting pulmonary vasoconstriction can be blocked by cyclooxygenase inhibitors (35). Furthermore, the rise in pulmonary vascular resistance is associated with increased thromboxane B_2 levels in sheep lung lymph (36–38). Thromboxane also appears to be the important mediator in a model of activated platelet-induced pulmonary hypertension (39). Our studies demonstrate that exposure to O_2 metabolites may be another mechanism that could stimulate the production of vasoactive arachidonate products within the lung and result in pulmonary hypertension.

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References

1. Brigham, K. L. 1982. Mechanisms of lung injury. *In* Clinics in Chest Medicine. R. C. Bone, editor. W. B. Saunders Co., Philadelphia. 3:9–24.

2. Hyers, T. M. 1981. Pathogenesis of adult respiratory distress syndrome: current concepts. *Semin. Resp. Med.* 2:104–107.

3. Shasby, D. M., K. M. VanBenthuysen, R. M. Tate, S. S. Shasby, I. F. McMurtry, and J. E. Repine. 1982. Granulocytes mediate acute edematous lung injury in rabbit lungs perfused with phorbol myristate acetate: role of oxygen radicals. *Am. Rev. Respir. Dis.* 125:443–447.

4. Heflin, A. J., and K. L. Brigham. 1981. Prevention by granulocyte depletion of increased vascular permeability of sheep lung following endotoxemia. *J. Clin. Invest.* 68:1253–1260.

5. Flick, M. R., J. Hoeffel, and N. C. Staub. 1981. Superoxide dismutase prevents increased lung vascular permeability after air emboli in unanesthetized sheep. *Fed. Proc.* 40:405. (Abstr.)

6. Johnson, K. J., J. C. Fantone, J. Kaplan, and P. A. Ward. 1981. In vivo damage of rat lungs by oxygen metabolites. *J. Clin. Invest.* 67:983–993.

7. Johnson, K. J., and P. A. Ward. 1981. Role of oxygen metabolites in immune complex injury of lung. *J. Immunol.* 126:2365–2369.

8. Martin, W. J., J. E. Gadek, G. W. Hunninghake, and R. G. Crystal. 1981. Oxidant injury of lung parenchymal cells. *J. Clin. Invest.* 68:1277–1288.

9. Zimmerman, G. A., A. D. Renzetti, and H. R. Hill. 1983. Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 127:290–300.

10. Tate, R. M., and J. E. Repine. 1983. Neutrophils and the adult respiratory distress syndrome: state of the art. *Am. Rev. Respir. Dis.* 128:552–559.

11. Tate, R. M., K. M. VanBenthuysen, D. M. Shasby, I. F. McMurtry, and J. E. Repine. 1982. Oxygen-radical-mediated permeability edema and vasoconstriction in isolated perfused rabbit lungs. *Am. Rev. Respir. Dis.* 126:802–806.

12. Tappel, A. L. 1973. Lipid peroxidation damage to cell components. *Fed. Proc.* 32:1870–1874.

13. Piper, P., and J. Vane. 1971. The release of prostaglandins from lung and other tissues. *Ann. NY Acad. Sci.* 180:363–385.

14. Hansen, T. R., and D. F. Bohr. 1975. Hypertension, transmural pressure and vascular smooth muscle response in rats. *Circ. Res.* 36:590–598.

15. Morris, H. G., N. A. Sherman, and F. T. Shepperdson. 1981. Variables associated with radioimmunoassay of prostaglandins in plasma. *Prostaglandins.* 21:771–788.

16. Curnutte, J. T., D. M. Whitten, and B. M. Babior. 1974. Defective superoxide production by granulocytes from patients with chronic granulomatous disease. *N. Engl. J. Med.* 290:593–597.

17. Scheffe, H. 1953. A method for judging all contrasts in the analysis of variance. *Biometrika.* 40:87–104.

18. Moncada, S., S. Bunting, K. Mullane, P. Thorogood, J. R. Vane, A. Raz, and P. Needleman. 1977. Imidazole: a selective inhibitor of thromboxane synthetase. *Prostaglandins.* 13:611–618.

19. Panganamala, R. V., H. M. Sharma, H. Sprecher, J. C. Geer, and D. G. Cornwell. 1974. A suggested role for hydrogen peroxide in the biosynthesis of prostaglandins. *Prostaglandins.* 8:3–11.

20. Panganamala, R. V., N. R. Brownlee, H. Sprecher, and D. G. Cornwell. 1974. Evaluation of superoxide anion and singlet oxygen in the biosynthesis of prostaglandins from eicosa-8, 11, 14-trienoic acid. *Prostaglandins.* 7:21–28.

21. Kellog, E. W., and I. Fridovich. 1975. Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. *J. Biol. Chem.* 250:8812–8817.

22. Lands, W. E. M., J. Sauter, and G. W. Stone. 1978. Oxygen requirement for prostaglandin biosynthesis. *Prostaglandins Med.* 1:117–120.

23. Lands, W. E. M. 1979. The biosynthesis and metabolism of prostaglandins. *Ann. Rev. Physiol.* 41:633–652.

24. Fridovich, S. E., and N. A. Porter. 1981. Oxidation of arachidonic acid in micelles by superoxide and hydrogen peroxide. *J. Biol. Chem.* 256:260–265.

25. Hemler, M. E., H. W. Cook, and W. E. M. Lands. 1979. Prostaglandin biosynthesis can be triggered by lipid peroxides. *Arch. Biochem. Biophys.* 193:340–345.

26. Hemler, M. E., G. Graff, and W. E. M. Lands. 1978. Accelerative autoactivation of prostaglandin biosynthesis by PGG_2. *Biochem. Biophys. Res. Commun.* 85:1325–1331.

27. Bakhle, Y. S. 1981. Biosynthesis of prostaglandins and thromboxanes in lung. *Bull. Eur. Physiopath. Respir.* 17:491–508.

28. Hamberg, M., J. Svensson, and B. Samuelsson. 1974. Prostaglandin endoperoxides: a new concept concerning the mode of action and release of prostaglandins. *Proc. Natl. Acad. Sci. USA.* 71:3824–3828.

29. Smedegard, G., P. Hedqvist, S. Dahlen, B. Revenase, S. Hammarstrom, and B. Samuelsson. 1982. Leukotriene C4 affects pulmonary and cardiovascular dynamics in monkey. *Nature (Lond.).* 295:327–329.

30. Froelich, J. C., M. L. Ogletree, B. Peskar, and K. L. Brigham. 1980. *Adv. Prostaglandin Thromboxane Res.* 7:745–750.

31. Watkins, W. D., P. C. Huttemeier, D. Kong, and M. B. Peterson. 1982. Thromboxane and pulmonary hypertension following E. coli endotoxin infusion in sheep: effect of an imidazole derivative. *Prostaglandins.* 23:273–285.

32. Ogletree, M. L., and K. L. Brigham. 1981. Imidazole, a selective inhibitor of thromboxane synthesis inhibits pulmonary vascular responses to endotoxin in awake sheep. *Am. Rev. Respir. Dis.* 123:247. (Abstr.)

33. Demling, R. H., M. Smith, R. Gunther, J. T. Flynn, and M. H. Gee. 1981. Pulmonary injury and prostaglandin production during endotoxemia in conscious sheep. *Am. J. Physiol.* 240:348–353.

34. Hales, C. A., L. Sonne, M. Peterson, D. Kong, M. Miller, and W. D. Watkins. 1981. Role of thromboxane and prostacyclin in pulmonary vasomotor changes after endotoxin in dogs. *J. Clin. Invest.* 68:497–505.

35. Ogletree, M. L., and K. L. Brigham. 1982. Effects of cyclooxygenase inhibitors on pulmonary vascular responses to endotoxin in unanesthetized sheep. *Prostaglandins, Leukotrienes Med.* 8:489–502.

36. Huttemeier, P. C., W. D. Watkins, M. B. Peterson, and W. M. Zapol. 1982. Acute pulmonary hypertension and lung thromboxane release after endotoxin infusion in normal and leukopenic sheep. *Circ. Res.* 50:688–694.

37. Smith, M. E., R. Gunther, M. Gee, J. Flynn, and R. H. Demling. 1981. Leukocytes, platelets, and thromboxane A2 in endotoxin-induced lung injury. *Surgery (St. Louis).* 90:102–107.

38. Demling, R. H. 1982. Role of prostaglandins in acute pulmonary microvascular injury. *Ann. NY Acad. Sci.* 384:517–534.

39. Heffner, J. E., S. A. Shoemaker, E. M. Canham, M. Patel, I. F. McMurtry, H. G. Morris, and J. E. Repine. 1983. Acetyl glyceryl ether phosphorylcholine-stimulated human platelets cause pulmonary hypertension and edema in isolated rabbit lungs: role of thromboxane A2. *J. Clin. Invest.* 71:351–357.