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Reactive Oxygen Metabolites Produce Pulmonary Vasoconstriction in Young Pigs

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ABSTRACT. Reactive oxygen metabolites appear to modulate pulmonary vascular changes. To study the effects of free radical formation *in vivo*, we investigated five groups of young pigs by recording hemodynamic changes after xanthine oxidase infusion alone and after pretreatment with hypoxanthine or possible blocking agents. The pulmonary vascular pressure increased rapidly in the groups without inhibition reaching maximum levels 25 min after the start of the experiment. The pulmonary artery blood flow declined toward minimum values at the same time. Compared to baseline levels, the calculated vascular lung resistance increased by 300% when the pigs were pretreated with hypoxanthine, and by 150% when xanthine oxidase was given alone. These findings suggest enhanced pulmonary vasoconstriction as a result of high initial hypoxanthine levels probably capable of forming larger quantities of oxygen radicals. The vascular reaction was attenuated when the pigs were pretreated with indomethacin (cyclooxygenase inhibitor) or allopurinol (xanthine oxidase inhibitor). Furthermore, the presence of catalase (hydrogen peroxide scavenger) reduced the pulmonary vasoconstriction significantly. We observed less decline in arterial oxygen tension and oxygen saturation when the animals had been pretreated with inhibitory agents, compared to the blood gas changes found in the xanthine oxidase group. The systemic pressure recordings in the carotid artery remained at baseline levels in all groups. We conclude that oxygen radicals formed by the hypoxanthine-xanthine oxidase system produce severe pulmonary vascular constriction in young pigs. (*Pediatr Res* 29: 543–547, 1991)

Abbreviations

Hx, hypoxanthine
PVR, pulmonary vascular resistance
XO, xanthine oxidase
Pa_{O_2}, arterial oxygen tension

The complex mechanisms governing the pulmonary circulation in the perinatal period have recently been given extensive consideration in several review articles (1–3). Oxygen-derived free radicals play an important role in experimentally induced hypoxic-reoxygenation injury (4, 5), and may be responsible for both hypoxic and hyperoxic aspects of pulmonary damage (6–8). One source of oxidant genertion in the lungs may be the Hx-XO system (6). The three reactive intermediates formed during successive univalent reductions of the oxygen molecule, the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH\cdot$), seem to exert different degrees of cytotoxicity (9–11). The pathologic influence of these metabolites is opposed by the antioxidant defense mechanisms of the lung, including prevention of radical formation, scavenging mechanisms, removal of oxidants from vital cellular structures, and the repair of molecular injury (12).

Recent experiments point out that oxygen radicals alter smooth muscle tone differently in different vascular beds. In the isolated lamb, ductus arteriosus relaxation occurred, the reaction mediated by arachidonic acid metabolites (13), and several investigators have reported systemic vasodilatation caused by oxygen intermediates (14, 15).

The pulmonary vascular reaction after O_2 radical exposure seems complex and controversial. In the isolated rabbit lung, vasoconstriction was found. This could be explained by increased cyclooxygenase metabolite formation, but was independent of the superoxide radical (16). In isolated perfused rat lungs, hypoxanthine-xanthine oxidase-induced radicals attenuate the vascular tone caused by hypoxia (17, 18). Moreover, the pulmonary edema in such lung preparations was formed by hydrogen peroxide, independent of cyclooxygenase products (19).

In the present study, we wanted to further investigate the aspects of oxygen radical formation in the lungs. We examined the hemodynamic consequences in young pigs when XO was given alone or combined with Hx. Subsequently, we studied the vascular reactions after pretreatment with possible inhibitory pharmacologic agents.

MATERIALS AND METHODS

Animal model. Thirty-eight young pigs of either sex weighing 13–23 kg (mean weight 19.3 ± 1.8 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (25 mg/kg), followed by 1–2 mg/kg every 30 min or as needed. Ringer acetate (50 mL/kg/h) was given i.v. from the start of surgery throughout the experiments, and a suprapubic bladder catheter was established for diuretic control. Pigs were tracheotomized and ventilated with a volume-controlled respirator receiving room air (Servo model 900 B, Elema-Schoenander, Stockholm, Sweden). Ventilator settings were regulated according to frequent blood gas determinations, and frequency and tidal volume adjusted to keep arterial CO_2 tension in the range of 3.75 to 4.25 kPa. Further ventilator regulations were not made after the start of the experiment. Rectally monitored body temperature was kept constant with a heating pad.

Catheters were positioned in the right atrium through the right external jugular vein for administration of pharmacologic agents, and in the left common carotid artery for systemic blood pressure recordings. A sternotomy was performed and catheters inserted into the pulmonary artery for pressure registrations (PAP) and

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into the left atrium for blood sampling and pressure registrations (LAP). All catheters were connected to transducers (Senso-Nor 840; Senso-Nor, Horten, Norway), and blood pressures registered on a Gould 2600s recorder (Gould Instruments Division, Cleveland, OH). Pulmonary vascular pressure = PAP − LAP. Pulmonary artery blood flow was recorded with an electromagnetic flowmeter, the probe being fitted around the proximal part of the pulmonary artery (Nycotron Flowmeter 373-S, Oslo, Norway). Pressure and flow recordings were registered continuously and the PVR calculated in SI units (dyne·s·cm^{-5}).

Experimental design. Five groups of pigs were studied. After completion of surgical procedures, a 30-min period was allowed for hemodynamic stabilization before baseline blood pressure and blood flow values were recorded. All animals received a bolus injection of 1.0 U/kg XO, diluted in Ringer acetate, 1.0 mL/kg body weight, at the start of the experiment. The injection was completed in 120 s. The pigs were studied for a minimum of 75 min, and arterial blood gas measurements, pH determination, and blood samples for biochemical analysis were taken at 5-, 10-, or 20-min intervals starting with baseline values. Blood gases were analyzed by an AVL 945 Automatic Blood Gas System (AVL Biomedical Instruments, Schaffhausen, Switzerland).

Experimental groups. Group 1 (n = 11) received XO alone. Group 2 (n = 5) was given Hx, 10 mmol/L, in Ringer-acetate, 10 mL/kg, from −30 to −20 min, and XO. Group 3 (n = 10) received indomethacin, 5.0 mg/kg bolus i.v. 18 h before surgery, and a further 2.5 mg/kg at −120 min, and XO. Group 4 (n = 6) was given allopurinol, 50 mg/kg from −30 to −20 min, and XO. Group 5 (n = 6) was given catalase, 25 000 U/kg, in Ringer acetate, 2.5 mL/kg as infusion from −60 to 60 min, and XO.

Pigs showing clinical signs of pneumonia, pericarditis, or hypoxemia were excluded before XO was infused. Sham-operated animals (n = 3) received Ringer acetate instead of XO and inhibitory agents. This group remained at hemodynamic and biochemical baseline levels throughout the experiments.

Reagents. XO (grade III, buttermilk: 1–2 U/mg protein), Hx (6-hydroxy-purine, crystalline), allopurinol (20 mg/mL prepared in 9 mg sodium chloride, 152 mg sodium hydroxide at a concentration of 1 mol/L, and sterile H_2O), and catalase (20 800 U/mg protein) were all obtained from Sigma Chemical Company (St. Louis, MO). Additional XO (from cow milk, 1 U/mg protein) was supplied by Boehringer Mannheim, Mannheim, Germany. Indomethacin (5 mg/mL) used in the sodium salt form came from Dumex Company, Copenhagen, Denmark.

The sp act of various XO batches from Sigma Chemical Company have been shown to vary quite widely *in vitro* (20). In the present experiments, we did not register statistically significant differences between the XO batches purchased from Sigma Chemical and Boehringer, either *in vitro* or *in vivo*. Enzyme activity was identical after vehicles had been removed by filtration through a Sephadex 25 column (Pharmacia LKB Biochemicals, Uppsala, Sweden). To test the vehicle of XO, ammonium sulphate in equal amounts and concentration as given with the commercial XO [2.3 M $(NH_4)_2SO_4$] was injected into the pulmonary circulation of two pigs. No effects on the pulmonary flow and pressure were recorded. In the same manner, XO without ammonium sulphate was given to two other pigs. Identical recordings with the commercial XO were found. For these reasons, we used unaltered commercial XO in all experiments.

Before use, XO and catalase were analyzed according to previously described methods to exclude disturbing protease and endotoxin activity (21, 22). The Sigma XO enzyme contained 4607 U/L kallikrein, 1297 U/L plasmin, 503 U/L trypsin (S-2222), 748 U/L trypsin (S-2677), and 1.04×10^4 ng/L endotoxin. The catalase contained 0 U/L protease and 3×10^5 ng/L endotoxin. These activities were considered to be of negligible effect in the doses applied.

The results presented are based on the XO group, including six animals given Sigma XO and five animals with enzyme from the Boehringer Company.

Hx analysis. Hx levels in plasma were analyzed according to a HPLC method described in former studies (23, 24). Plasma samples were prepared by centrifugation for 30 min through a filter with 30.000 molecular wt cut-off (YMT-filter, MPS-1 System; Amicon Corp., Danvers, MA). The chromatography was performed on a reverse-phase column (0.45 × 15 cm) of 5-μm material (Pecosphere-5C C18, Perkin Elmer, Norwalk, CT). The eluting compounds were detected at 254 nm. Identification of the peaks in the HPLC effluent was made on the basis of retention times. These were compared with retention times of known pure standards measured routinely after every fourth sample. Peak height ratios at 254 *versus* 280 nm were measured and small variations in retention times or double peaks were checked by coelution with standard solutions. Quantification of peaks was performed by peak height ratios. Standard solutions were purchased from Sigma Chemical Company.

Statistics. Statistical analysis of the data was performed by the Wilcoxon two-sample rank sum test, significance level $p < 0.05$. Values are expressed as arithmetic mean ± SD.

RESULTS

Baseline measurements. Table 1 lists the hemodynamic baseline values after the stabilization, but before infusion, for all groups. The pulmonary pressure in the Hx plus XO group was lower than in the XO group ($p < 0.05$) and the PVR differed statistically compared to this group ($p < 0.01$). During the Hx infusion in this group, from time −30 to −20 min, the PVR increased from the baseline value of 448 ± 89 to 503 ± 152 dyne·s·cm^{-5} (NS) before reaching a steady preexperimental value of 473 ± 119 dyne·s·cm^{-5}. At time zero, however, no statistical differences between the Hx plus XO group and the XO group could be found.

In the sham-operated animals (n = 3), the hemodynamic parameters remained at baseline levels throughout the observation period of 80 min. The pulmonary pressure increased from 13.0 ± 1.0 to 14.0 ± 2.0 mm Hg after 25 min (NS), declining to 13.7 ± 2.1 mm Hg after 80 min (NS). The blood flow variation in this group was similarly negligible, 90.0 ± 2.6 mL/kg/min at baseline levels *versus* 89.5 ± 2.7 (NS) and 87.1 ± 0.7 mL/kg/min (NS) at the 25- and 80-min mark respectively. The PVR increased slightly, but not significantly, during the experiments, from 651 ± 141 dyne·s·cm^{-5} before the start to 688 ± 133 (NS) after 25 min and 693 ± 159 (NS) after 80 min.

Oxygenation. Table 2 gives absolute values for Pa_{O_2} (kPa) and oxygen saturation (%) at baseline levels; at 25 min after XO administration, when maximum PVR was recorded in all groups; and after the end of the 80-min observation period. The baseline values were recorded after the carotid artery had been catheterized, but before inhibitory substances or Hx were infused. The pigs in the allopurinol group had a mean 14% higher baseline Pa_{O_2} than the XO group ($p < 0.05$). The other groups did not differ in baseline oxygen tension values. All the groups declined

Table 1. *Hemodynamic baseline measurements for all groups**

Group	▲P (mm Hg)	PAQ (mL/kg/min)	PVR (dyne·s·cm^{-5})
XO alone	14.0 ± 1.7	96.3 ± 19.8	650 ± 129
Hx + XO	10.8 ± 2.4†	87.4 ± 6.8	448 ± 89‡
Indo + XO	15.5 ± 3.3	104.4 ± 15.5	604 ± 108
Allo + XO	15.8 ± 2.2	99.5 ± 14.2	680 ± 101
Cat + XO	13.0 ± 1.7	95.8 ± 8.5	588 ± 76

* Absolute values are mean ± SD. ▲P, pulmonary pressure; PAQ, pulmonary artery blood flow; Indo, indomethacin; Allo, allopurinol; Cat, catalase.

† $p \leq 0.05$ compared to XO group.

‡ $p \leq 0.01$ compared to XO group.

Table 2. *Arterial blood gases and oxygen saturation at baseline levels, at PVR maximum after 25 min, and at end of 80-min observation period**

	Group	Baseline	25 min	80 min
Pao_2 (kPa)	XO alone	11.20 ± 1.60	5.81 ± 1.57	6.56 ± 1.68
	Hx + XO	11.46 ± 0.80	6.92 ± 1.17	8.27 ± 2.30
	Indo + XO	11.56 ± 1.31	8.17 ± 1.27†	7.34 ± 1.13
	Allo + XO	12.80 ± 0.71‡	9.31 ± 2.15†	8.77 ± 1.68§
	Cat + XO	12.51 ± 1.16	9.92 ± 1.75†	9.43 ± 2.08§
SAT O_2 (%)	XO alone	96.9 ± 1.7	80.8 ± 8.6	84.9 ± 8.7
	Hx + XO	97.0 ± 1.0	87.8 ± 6.2	90.3 ± 8.9
	Indo + XO	97.0 ± 1.2	91.7 ± 4.5†	89.0 ± 4.7
	Allo + XO	97.5 ± 0.6	94.9 ± 2.3†	94.0 ± 2.8§
	Cat + XO	97.3 ± 0.7	95.4 ± 1.5†	94.0 ± 3.6§

* Values are mean ± SD. SAT O_2, oxygen saturation; Indo, indomethacin; Allo, allopurinol; CAT, catalase.
† $p \leq 0.01$ compared to XO group.
‡ $p \leq 0.05$ compared to XO group.
§ $p \leq 0.02$ compared to XO group.

significantly lower than baseline levels at both 25 and 80 min ($p < 0.05$ in inhibitory groups, $p < 0.01$ in the XO and Hx groups).

We found significantly lower Pao_2 and oxygen saturation decline in all groups given inhibitors compared with the XO group ($p < 0.01$). The allopurinol and catalase groups also differed from the XO group after 80 min ($p < 0.02$) as well. pH levels were not affected in any group (results not shown).

Plasma Hx concentrations. The Hx levels in plasma were measured at regular intervals before and after XO infusion. In the group pretreated with Hx, the −5 min baseline value of Hx was 89.5 ± 34.9 μmol/L, whereas all other groups had a common mean −5 min hypoxanthine value of 18.9 ± 5.8 μmol/L (mean ± SD). This level is in accordance with Hx measurements from an earlier study in nonhypoxemic porcine plasma (25), and we did not find significantly different Hx concentrations between the four groups not treated with Hx. Five min after the XO infusion, Hx, as expected, was virtually cleared from the circulation, except in the group pretreated with the XO inhibitor allopurinol. In this group, Hx concentrations at 5 min remained unchanged from baseline values (20.4 ± 4.3 μmol/L). In the sham-operated animals, the Hx levels also kept constant throughout the experiments (20.8 ± 0.8 μmol/L).

Blood pressures and flow. Systemic blood pressure recordings in the carotid artery showed a slight, but insignificant rise after the XO infusion in the Hx and XO groups. Such variations were not observed in the groups given inhibitory pharmacologic agents (data not shown). During catalase infusion, the pulmonary artery pressure increased from 20.0 ± 1.3 mm Hg until a steady baseline level of 26.8 ± 3.1 mm Hg ($p < 0.01$) was reached 20 min before the XO infusion. When allopurinol was given, we recorded a temporary insignificant drop in the pulmonary artery pressure. In the other groups, no changes in pulmonary artery blood pressure or pulmonary artery blood flow were recorded between baseline and time zero values.

Blood pressures registered in the left atrium remained at baseline levels throughout the experiments in all animals, and blood flow measurements were not affected by infusion of inhibitory substances.

Pulmonary circulation. All animals had stable baseline pressure and flow recordings in the pulmonary artery when XO was given at time zero. Figure 1 shows the relative increase in pulmonary pressure during the experiments in the groups given XO alone and after pretreatment with Hx or indomethacin. In the XO group, the pressure increase from time zero to 25 min was 18.5 ± 6.4 mm Hg compared with 25.0 ± 6.9 mm Hg for the Hx plus XO group (NS). In the group pretreated with indomethacin, the pressure rise was 4.2 ± 6.7 mm Hg ($p < 0.01$), significantly lower than in the XO group. Figure 2 gives the relative rise in pulmonary pressure from time zero to 25 min for all groups. The

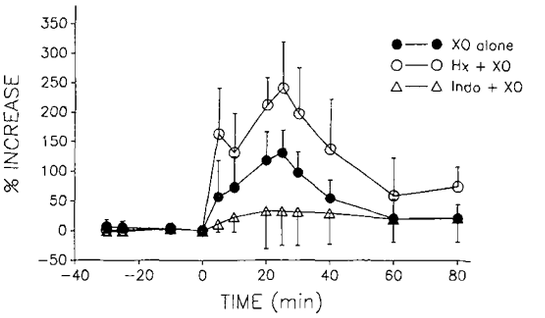

Fig. 1. Relative increase in pulmonary pressure with time after infusion of 1 U/kg XO at time zero in the groups given XO alone ($n = 11$), Hx + XO ($n = 5$), and indomethacin (*Indo*) before XO ($n = 10$). Values are mean ± SD.

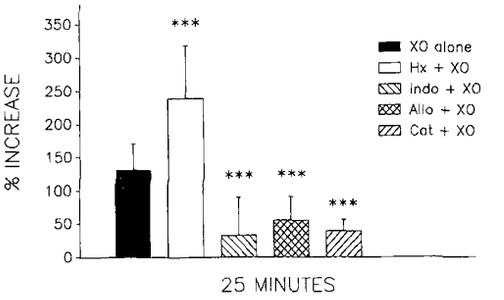

Fig. 2. Relative increase in pulmonary pressure compared to baseline levels 25 min after infusion of 1 U/kg XO. Groups were given XO alone ($n = 11$), Hx + XO ($n = 5$), indomethacin (*Indo*) + XO ($n = 10$), allopurinol (*Allo*) + XO ($n = 6$), or catalase (*Cat*) + XO ($n = 6$). Values are mean ± SD. ***, $p < 0.01$ compared with XO group.

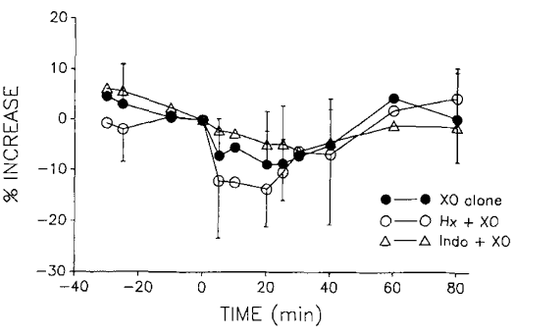

Fig. 3. Relative increase in pulmonary artery flow with time, after infusion of 1 U/kg XO at time zero in pigs given XO alone ($n = 11$), Hx + XO ($n = 5$), and indomethacin (*Indo*) before XO ($n = 10$). Values are mean ± SD.

increase in allopurinol-treated animals was 7.5 ± 4.0 mm Hg ($p < 0.01$), significantly lower than the XO group. In the catalase group, the pulmonary pressure rise was 7.8 ± 3.3 mm Hg ($p < 0.01$), significantly lower than the XO group.

The relative decline in pulmonary blood flow from zero values is presented in Figures 3 and 4. In the XO group, the flow at 25 min was reduced by 8.3 ± 5.0 mL/kg/min, and blood flow in the groups given Hx and XO and the group pretreated with indomethacin before XO was lowered by 9.3 ± 4.9 and 4.3 ± 6.1 mL/kg/min, respectively (NS compared with decrease in the XO group). In the group pretreated with allopurinol, the flow diminished by 4.7 ± 7.2 mL/kg/min (NS compared with XO), and in the catalase group, by 2.4 ± 5.9 mL/kg/min, significantly less than in the XO group ($p < 0.05$).

Figure 5 describes the relative increase from zero values in PVR in the XO group, the Hx plus XO group, and the group pretreated with indomethacin.

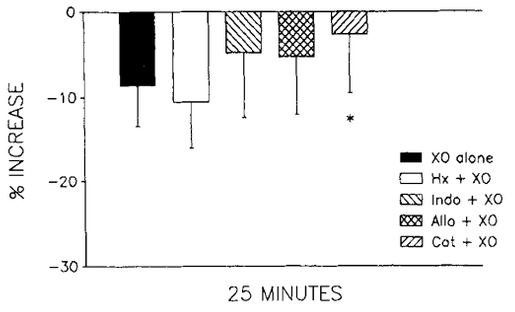

Fig. 4. Relative increase in pulmonary artery flow compared to baseline levels 25 min after infusion of 1 U/kg XO. Groups were given XO alone (n = 11), Hx + XO (n = 5), indomethacin (*Indo*) before XO (n = 10), allopurinol (*Allo*) + XO (n = 6), or catalase (Cat) + XO (n = 6). Values are mean ± SD. *, $p < 0.05$ compared with XO group.

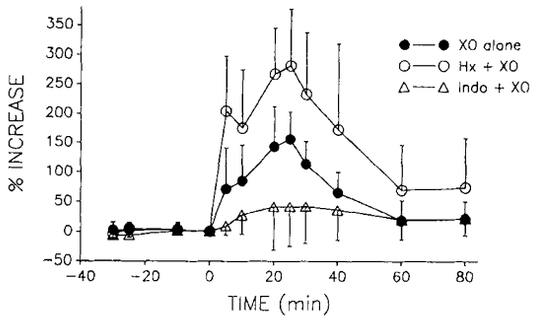

Fig. 5. Relative increase in PVR with time after infusion of 1 U/kg XO at time zero. Groups were given XO alone (n = 11), Hx + XO (n = 5), or indomethacin (*Indo*) + XO (n = 10). Values are mean ± SD.

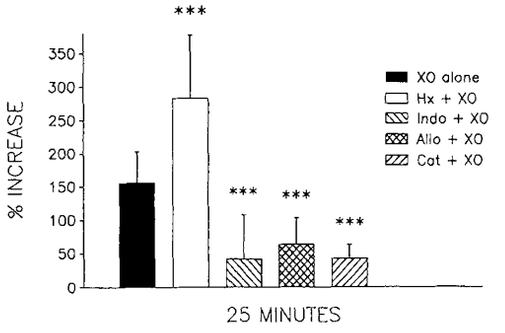

Fig. 6. Relative increase in PVR compared to baseline levels 25 min after infusion of 1 U/kg XO. Groups were given XO alone (n = 11), Hx + XO (n = 5), indomethacin (*Indo*) + XO (n = 10), allopurinol (*Allo*) + XO (n = 6), or catalase with XO (n = 6). Values are mean ± SD. ***, $p < 0.01$ compared with XO group.

Notable is the immediate PVR rise in the Hx plus XO group. Five min after XO administration, the resistance in this group was 994 ± 558 dyne·s·cm^{-5} compared to 463 ± 460 dyne·s·cm^{-5} in the XO group ($p < 0.05$).

Figure 6 addresses the relative increase in PVR at 25 min. Peak values were recorded in all groups at this time. The XO-treated animals had a PVR increase of 1008 ± 431 *versus* 1284 ± 356 dyne·s·cm^{-5} for the Hx plus XO group. In the group pretreated with indomethacin, the resistance increased 275 ± 404 dyne·s·cm^{-5}, significantly lower than in the XO group ($p < 0.05$), and in allopurinol and the catalase groups, the rise in PVR was 376 ± 215 and 436 ± 249 dyne·s·cm^{-5}, respectively ($p < 0.01$ *versus* XO).

DISCUSSION

Our results show that oxygen metabolites generated by the Hx-XO reaction potently constrict the pulmonary circulation in young pigs. The formation of free radicals in this reaction has been well documented in earlier studies (10, 11, 26, 27), and it has been used to investigate vasoactive properties in various animal models (13–15).

In recent years, considerable evidence has accumulated indicating pulmonary damage due to oxygen metabolite formation. The mechanisms by which they exert their action, and to what extent either one of the radicals participate, have not yet been clarified (6, 8, 28).

In this study, we found a rapid increase in the pulmonary vascular pressure after XO infusion both when XO was given alone and in combination with Hx. There was a simultaneous decrease in blood flow and, therefore, an undisputable increase in PVR. In the group pretreated with Hx, the relative PVR increase at 5 and 25 min was significantly higher than in the control group given XO alone. This suggests that an increased radical production commences when the initial Hx substrate level is elevated.

The increased PVR was blocked by indomethacin, a cyclooxygenase inhibitor, suggesting activation of the arachidonic acid metabolism. Our observation is in agreement with the work of Tate *et al.* (16) who found increased mean pulmonary artery perfusion pressure induced by O_2 metabolites in isolated rabbit lungs. This group showed that the pressure augmentation was induced by thromboxane generation. The reaction was blocked by various inhibitors along the cyclooxygenase pathway in the arachidonic acid cascade.

The PVR increase in our study was attenuated by catalase, indicating that the reaction was initiated either by hydrogen peroxide or a H_2O_2-derived product, such as the hydroxyl radical. Our results are consistent with earlier observations (16, 29). In these studies, catalase completely blocked the pulmonary vasoconstriction induced by XO.

In a recent investigation by Seeger *et al.* (30), hydrogen peroxide was presented to the pulmonary artery of isolated perfused rabbit lungs. The study showed a rapid vasoconstrictive response accompanied by thromboxane and prostacyclin release, which was blocked by indomethacin. Our results seem to support their findings.

The present results show that the increased pulmonary resistance produced by the Hx-XO system mainly affects the pulmonary vascular pressure. The generally insignificant decrease in blood flow in the different animal groups seems to be of less importance. The relatively impaired cardiac output indicates that in our porcine model, the generated O_2 metabolites act mainly in the pulmonary vascular bed. This is in contrast to what Prasad *et al.* (31) found when they studied cardiovascular changes induced by the Hx-XO system in a canine model. They observed increased PVR, but in their investigation the cardiac output was reduced, suggesting a compromised heart function after xanthine-XO infusion. They also found a transient decrease in mean pulmonary artery pressure, and the effects observed on pulmonary hemodynamics were not significantly altered by pretreatment with superoxide dismutase and catalase combined. In that study, the free radical scavengers were given as a bolus dose 5 min before the start of the experiments. Considering the rapid turnover of these enzymes (32) and the small amounts applied, this mode of administration may have given insufficient scavenging effect.

In our investigation, pretreatment with allopurinol gave excellent protection against the increase in PVR. Allopurinol acts through one of three different mechanisms: *1*) as a substrate for XO competing with Hx and xanthine by forming oxypurinol (33); *2*) through a direct scavenging effect against oxygen radicals (34); or *3*) by a combination of these two mechanisms (34).

In earlier reports from comparable investigations, blood gas changes have been given little consideration. One study did not show any significant changes in pH and Pa_{O_2} between time zero measurements and the 60-min observation (31). We registered less decline in arterial oxygen pressure and saturation at maxi-

mum PVR (25 min) after pretreatment with inhibitory agents ($p \leq 0.01$). At the end of the 80-min observation period, there were significant blood gas variations between the XO group and the groups inhibited with allopurinol and catalase ($p \leq 0.02$) (Table 1). These results support the findings of severely compromised lung function when young pigs are exposed to the Hx-XO system.

Our investigation demonstrates a highly significant rise in PVR, resulting in a dramatic increase in pulmonary vascular pressure and altered blood gases when porcine lungs are exposed to an O_2 radical-generating system. The hemodynamic changes seemed to be dependent on the Hx levels measured before XO was introduced. The hemodynamic changes and blood gas variations were reduced by the hydrogen peroxide scavenger catalase and the XO inhibitor allopurinol. The blocking effect of indomethacin indicates that metabolites from the cyclooxygenase pathway of the arachidonic acid cascade are activated in the reaction. The animals did not return to baseline levels during the observation period, suggesting persistent lung injury. Future investigations are needed to further enlighten the mechanisms behind this kind of pulmonary damage.

REFERENCES

1. Rudolph AM 1977 Fetal and neonatal pulmonary circulation. Am Rev Respir Dis 115:11–18
2. Reid LM 1979 The pulmonary circulation: remodeling in growth and disease. Am Rev Respir Dis 119:531–546
3. Cassin S 1987 Role of prostaglandins, thromboxanes and leukotrienes in the control of the pulmonary circulation in the fetus and newborn. Semin Perinatol 11:53–63
4. McCord JM 1985 Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312:159–163
5. Saugstad OD 1988 Hypoxanthine as an indicator of hypoxia: its role in health and disease through free radical production. Pediatr Res 23:143–150
6. Saugstad OD 1985 Oxygen radicals and pulmonary damage. Pediatr Pulmonol 1:167–175
7. Saugstad OD, Hallman M, Abraham JL, Epstein B, Cochrane C, Gluck L 1984 Hypoxanthine and oxygen induced lung injury: a possible basic mechanism of tissue injury? Pediatr Res 18:501–504
8. Freeman BA, Crapo JD 1981 Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. J Biol Chem 256:10986–10992
9. Crapo JD, Tierny DF 1974 Superoxide dismutase and pulmonary oxygen toxicity. Am J Physiol 226:1401–1407
10. Link EM, Riley PA 1988 Role of hydrogen peroxide in the cytotoxicity of the xanthine/xanthine oxidase system. Biochem J 249:391–399
11. Kuppusamy P, Zweier JL 1989 Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation. J Biol Chem 264:9880–9884
12. Heffner JE, Repine JE 1989 Pulmonary strategies of antioxidant defense. Am Rev Resp Dis 140:531–554
13. Clyman RI, Saugstad OD, Mauray F 1989 Reactive oxygen metabolites relax the lamb ductus arteriosus by stimulating prostaglandin production. Circ Res 64:1–8
14. Rosenblum WI 1983 Effects of free radical generation on mouse pial arterioles: probable role of hydroxyl radicals. Am J Physiol 245:H139–H142
15. Kontos HA 1985 Oxygen radicals in cerebral vascular injury. Circ Res 57:508–516
16. Tate RM, Morris HG, Schroeder WR, Repine JE 1984 Oxygen metabolites stimulate thromboxane production and vasoconstriction in isolated saline-perfused rabbit lungs. J Clin Invest 74:608–613
17. Weir EK, Eaton JW, Chesler E 1985 Redox status and pulmonary vascular reactivity. Chest 88:249S–252S
18. Archer SL, Peterson D, Nelson DP, DeMaster EG, Kelly B, Eaton JW, Weir EK 1989 Oxygen radicals and antioxidant enzymes alter pulmonary vascular reactivity in the rat lung. J Appl Physiol 66:102–111
19. Burghuber O, Mathias MM, McMurtry IF, Reeves JT, Voelkel NF 1984 Lung edema due to hydrogen peroxide is independent of cyclooxygenase products. J Appl Physiol 56:900–905
20. Greenwald RA, Moy WW 1979 Inhibition of collagen gelation by action of the superoxide radical. Arthritis Rheum 22:251–255
21. Aasen AO, Kierulf P, Strømme JH 1982 Methodological considerations on chromogenic peptide substrate assays and application on automated analyzers. Acta Chir Scand 509:17–22
22. Aune S, Aasen AO, Kierulf P, Strømme JH 1982 Quantitative determination of endotoxin in plasma by means of a new chromogenic peptide substrate. A methodological study. Acta Chir Scand 509:55–57
23. Wung WE, Howell SB 1980 Simultaneous liquid chromatography of 5-fluorouracil, uridine, hypoxanthine, xanthine, uric acid, allopurinol, and oxypurinol in plasma. Clin Chem 26:1704–1708
24. Simmonds RJ, Harkness RA 1981 High-performance liquid chromatographic methods for base and nucleoside analysis in extracellular fluids and in cells. J Chromatogr 226:369–381
25. Saugstad OD, Aasen AO 1980 Plasma hypoxanthine concentration in pigs. A prognostic aid in hypoxia. Eur Surg Res 12:123–129
26. McCord JM, Fridovich I 1968 The reduction of cytochrome c by milk xanthine oxidase. J Biol Chem 243:5753–5760
27. Fridovich I 1970 Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. J Biol Chem 245:4053–4057
28. Johnson KT, Fantone III JC, Kaplan J, Ward PA 1981 *In vivo* damage of rat lungs by oxygen metabolites. J Clin Invest 67:983–993
29. Tate RM, Vanbenthuysen KM, Shasby DM, McMurty IF, Repine JE 1982 Oxygen-radical-mediated permeability edema and vasoconstriction in isolated perfused rabbit lungs. Am Rev Respir Dis 126:802–806
30. Seeger W, Suttorp N, Schmidt F, Neuhof H 1986 The glutathione redox cycle as a defense system against hydrogen-peroxide-induced prostanoid formation and vasoconstriction in rabbit lungs. Am Rev Respir Dis 133:1029–1036
31. Prasad K, Kalra J, Chan WP, Chaudhary AK 1989 Effect of oxygen free radicals on cardiovascular function at organ and cellular levels. Am Heart J 117:1196–1202
32. Viau AT, Abuchowski AA, Greenspan S, Davis FF 1986 Safety evaluation of free radical scavengers peg-catalase and peg-superoxide dismutase. J Free Radicals Biol Med 2:283–288
33. Spector T, Hall WW, Krenitsky TA 1986 Human and bovine xanthine oxidase: inhibition studies with oxipurinol. Biochem Pharmacol 35:3109–3114
34. Moorhouse CP, Grootveld M, Hallivell B, Quinlan GJ, Gutteridge JMC 1987 Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett 213:23–28