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Research Article

The effect of dazoxiben, a selective thromboxane (Tx) synthetase inhibitor, on systemic and pulmonary hemodynamics, eicosanoids, and lung permeability was assessed in awake goats with lung lymph fistulae following infusion of Escherichia coli endotoxin (1 microgram/kg). Animals received endotoxin either with no treatment or pretreatment with a bolus (25 mg/kg) followed by a maintenance infusion (10 mg/kg per h) of dazoxiben. In untreated animals, the peak rise of 26.8 cm H2O in pulmonary artery (Ppa) and of 13.5 cm H2O in wedge (Pw) pressures occurred at the same time as the peak elevations in plasma thromboxane B2 (T X B2). Maximum reduction in cardiac output (Qt) also occurred at the same time. Lung lymph flow (QL) increased during this period and remained elevated for at least 6 h after endotoxin. T X B2 levels had returned from a peak of 13.1 to 0.7 ng/ml by 2 h. In dazoxiben-treated animals, plasma concentrations of T X B2 were never significantly elevated. Increases in Ppa and Pw were markedly reduced and decreased Qt was transient. QL in treated animals began to increase by 30 min after endotoxin and reached a peak by 2 h. Increased QL in treated animals was not as great as in the untreated animals. Moreover, lymph-plasma protein ratios increased significantly in treated animals. Plasma prostaglandin (PG)F2 alpha and […]

Thromboxane A_2 Mediates Lung Vasoconstriction but Not Permeability after Endotoxin

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ABSTRACT The effect of dazoxiben, a selective thromboxane (Tx) synthetase inhibitor, on systemic and pulmonary hemodynamics, eicosanoids, and lung permeability was assessed in awake goats with lung lymph fistulae following infusion of *Escherichia coli* endotoxin (1 μg/kg). Animals received endotoxin either with no treatment or pretreatment with a bolus (25 mg/kg) followed by a maintenance infusion (10 mg/kg per h) of dazoxiben. In untreated animals, the peak rise of 26.8 cm H_2O in pulmonary artery (Ppa) and of 13.5 cm H_2O in wedge (Pw) pressures occurred at the same time as the peak elevations in plasma thromboxane B_2 (TxB_2). Maximum reduction in cardiac output ($\dot{Q}_t$) also occurred at the same time. Lung lymph flow ($\dot{Q}_L$) increased during this period and remained elevated for at least 6 h after endotoxin. TxB_2 levels had returned from a peak of 13.1 to 0.7 ng/ml by 2 h. In dazoxiben-treated animals, plasma concentrations of TxB_2 were never significantly elevated. Increases in Ppa and Pw were markedly reduced and decreased $\dot{Q}_t$ was transient. $\dot{Q}_L$ in treated animals began to increase by 30 min after endotoxin and reached a peak by 2 h. Increased $\dot{Q}_L$ in treated animals was not as great as in the untreated animals. Moreover, lymph-plasma protein ratios increased significantly in treated animals. Plasma prostaglandin (PG)$F_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ concentrations were elevated in both groups after endotoxin with values significantly greater in treated animals. We conclude that selective inhibition of Tx ameliorates many adverse hemodynamic consequences of endotoxemia but does not prevent lung permeability changes.

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INTRODUCTION

Gram-negative sepsis may be one of the causes of increased permeability and subsequent lung failure seen in patients with adult respiratory distress syndrome (1, 2). Infusion of endotoxin or live bacteria into awake animals has been shown to increase pulmonary artery pressure and pulmonary vascular permeability (3, 4), and in sufficient quantities, results in increased lung water (5). Endotoxin caused large elevations of aortic plasma thromboxane A_2(TxA_2),[1] prostacyclin (PGI_2) (6–9), and prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), but prostaglandin E_2(PGE_2) either did not increase (6–8, 10, 11) or it increased transiently (12, 13). Acute hemodynamic and permeability changes also accompanied endotoxemia. The eicosanoids have potent vasomotor effects with $PGF_{2\alpha}$ and TxA_2, causing vasoconstriction and PGI_2 vasodilation. Their relative amounts may be an important factor in the hemodynamic and permeability changes of endotoxemia.

Many studies have investigated the effect of cyclooxygenase inhibitors on the survival rate after endotoxin shock (14–21). These studies used acetylsalicylic acid, salicylate, indomethacin, ibuprofen, and meclofenamate as inhibitors. In general, survival was markedly improved in all of the species used (dogs, cats, rats, baboons). These studies with cyclooxygenase inhibitors, however, cannot define the precise role of individual eicosanoids, since synthesis of all of these were inhibited.

[1] *Abbreviations used in this paper:* CMN, caudal mediastinal lymph node; L/P, lymph-to-plasma protein ratio; Pa, systemic arterial pressure; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGH_2, prostaglandin endoperoxide; PGI_2, prostacyclin; Ppa, pulmonary artery pressure; Pv, central venous pressure; PVR, pulmonary vascular resistance; Pw, pulmonary wedge pressure; $\dot{Q}_L$, lung lymph flow; $\dot{Q}_t$, cardiac output; SVR, systemic vascular resistance; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

Recent studies have shown that dazoxiben (UK 37-248) is a specific inhibitor of thromboxane synthetase (22–25). The concentration that gave 50% inhibition of TxA_2 in an enzyme preparation was 2×10^{-9} M. When this concentration was increased by more than four orders of magnitude (1×10^{-4} M), there was still no inhibitory effect on the production of prostaglandin endoperoxide (PGH_2) and only a 30% inhibition of PGI_2 (24). At concentrations producing 100% inhibition of TxA_2 (5×10^{-7} M), there was a partial diversion of PGH_2 towards PGI_2, $PGF_{2\alpha}$, and PGE_2 (23). There was no change in hydroxyeicosatetraenoic acid (a lipoxygenase pathway product) when concentration was varied from 0.01 to 100 μmol/liter. These studies imply that dazoxiben does not block the synthesis of PGI_2, $PGF_{2\alpha}$, PGE_2, or the lipoxygenase products.

Watkins et al. (9) used an imidazole derivative (RO-22-3581, Hoffman-La Roche Inc., Nutley, NJ) that is a selective thromboxane synthetase inhibitor to demonstrate that TxA_2 mediates most of the pulmonary hypertension after endotoxin infusion. The role of TxA_2 on other hemodynamic and permeability changes, however, remains undefined. For this reason, we examined the effects of dazoxiben on hemodynamic and permeability changes after infusion of *Escherichia coli* endotoxin in goats.

METHODS

Animal preparation. Goats of mixed breeds were prepared with vascular catheters and a lymph fistula as previously described (26, 27). In one procedure, we cannulated the largest efferent duct of the caudal mediastinal lymph node (CMN) and then ligated all other efferent ducts. In the other preparation, a pouch was formed in the thoracic duct where the largest efferent duct of the CMN entered and then the pouch was cannulated. Care was taken to eliminate lymphatics entering the thoracic duct that did not come from the CMN. The CMN was then ligated and sectioned at the level of the inferior pulmonary ligament. Care was taken to insure that as many of the diaphragmatic and esophageal lymphatics as possible were ligated. These preparations have been shown to produce nearly pure lung lymph. Catheters were placed in the aorta and vena cava for pressure measurements and blood sampling. The day before the start of experiments, a Swan Ganz flow directed thermistor tipped catheter (Edwards Laboratories, Santa Ana, CA) was placed in the pulmonary artery for pressure measurement. Cardiac output ($\dot{Q}_t$) was computed by standard thermodilution technique (model 9520A, Edwards Laboratories).

Physiological measurements. We measured mean pulmonary artery (Ppa), pulmonary wedge (Pw) at end expiratory points, central venous (Pv), and systemic arterial pressures (Pa) and tabulated their values every 15 min. Lung lymph was collected into tared tubes for 15-min periods and lymph flows calculated by weighing. Blood was drawn into heparinized tubes every 30 min for plasma protein determinations. Total protein content of lymph and plasma was determined by a modified biuret technique (28), (Auto-Analyzer, Technicon Instruments Corp., Tarrytown, NY). Plasma protein concentration of blood drawn at the completion of a lymph collection period was used when appropriate for lymph/plasma protein ratio (L/P). If no blood was drawn, the concentration of plasma from the prior sample was used. $\dot{Q}_t$ was calculated every 30 min after 5-ml injections of iced saline. Endotoxin was not administered until at least 2 h of stable base line had been achieved. Therefore, $\dot{Q}_t$ was not always calculated at the same time relative to the start of the endotoxin infusion. Average values of $\dot{Q}_t$ were calculated every 15 min and when $\dot{Q}_t$ was not measured the previous determination was used for averaging. Pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) were calculated as $(Ppa - Pw)/\dot{Q}_t$ and $(Pa - Pv)/\dot{Q}_t$, respectively. On each of the experimental days we collected base-line data for 2 h to insure that the animals were in a steady state before any intervention.

Eicosanoid measurements. Blood for eicosanoid measurements was drawn from the aorta into 3-ml syringes containing 0.5 ml of acid-citrate-dextrose solution (NIH formula A) with 5 mM aspirin. Samples were then transferred to polypropylene tubes and spun at 35,000 *g* for 10 min at 4°C. Plasma was decanted, frozen, and maintained at −35°C.

Thromboxane B_2 (TxB_2) and 6-keto-$PGF_{1\alpha}$ concentrations were determined by radioimmunoassay kit with labeled tracers, standards, and antibodies from New England Nuclear, Boston, MA (NEK 007, NEK 008). Cross-reactivity of the TxB_2 antibody at 50% B/B_o was PGE_2 0.2%, PGA_2 < 0.2%, PGF_2 < 0.2%, and 6-keto-$PGF_{1\alpha}$ < 0.2% (New England Nuclear Technical Bulletin NEK-007). Cross-reactivity of the 6-keto-$PGF_{1\alpha}$ antibody at 50% B/B_o was $PGF_{1\alpha}$ 7.8%, PGE_1 3%, $PGF_{2\alpha}$ 2.7%, PGE_2 2%, PGA_1 < 0.3%, PGA_2 < 0.1%, TxB_2 < 0.1%, and 13,14 dihydro-15-keto-$PGF_{2\alpha}$ < 0.02% (New England Nuclear Technical Bulletin NEK-008). $PGF_{2\alpha}$ was measured by radioimmunoassay kit with labeled tracer, standards, and antibody from Seragen Inc., Boston, MA (56-6002). Cross-reactivity of the $PGF_{2\alpha}$ antibody at 50% B/B_o was TxB_2 0.3%, PGE_2 0.2%, PGD_2 < 0.2%, 6-keto-$PGF_{1\alpha}$ < 0.1%, PGA_2 < 0.1% (Seragen Technical Bulletin S-6600). The lower limit of detection was 0.1 ng/ml for TxB_2 and 6-keto-$PGF_{1\alpha}$ and 27 pg/ml for $PGF_{2\alpha}$. All samples were run with the same kit.

Eicosanoid levels were determined by direct assay of unextracted goat plasma samples. Matrix effects due to proteins present in unknown goat plasma samples were determined in eicosanoid-free plasma. Plasma was prepared for this purpose from pooled normal goat plasma obtained and processed identically as unknown plasma samples. The pooled normal goat plasma was then incubated with 50 mg/ml Norit A charcoal (Amend Drug and Chemical Co., Irvington, NJ) for 2 h with constant stirring. The charcoal was then removed by centrifugation and the charcoal-strip plasma filtered (0.2-μm filter, Millipore/Continental Water Systems, Bedford, MA) and frozen at −35°C. The standard curves for all three eicosanoids run in the eicosanoid-free plasma control were identical to those run in buffer alone (i.e., 95% B/B_o, 50% B/B_o, and 5% B/B_o differed by <10% between the two standard curves).

Experimental protocol. All of the studies were carried out in awake animals that were free to stand up or lie down and had free access to food and water. After control conditions were documented for a period of 2 h, endotoxin (*E. coli:* 055:B5, Sigma Chemical Co., St. Louis, MO) dissolved in sterile saline was infused intravenously over 30 min with a total dose of 1 μg/kg. 12 infusions were performed in six goats. Each goat received endotoxin with and without dazoxiben (provided by Dr. P. Urquilla, Pfizer, Inc., Groton, CT). Infusions in each animal were separated by 48 h to allow the animals time to recover from the endotoxin. Se-

quence of the experiments were alternated to insure that differences between treated and untreated animals were the result of dazoxiben and not the experimental protocol. Control untreated animals received only an endotoxin infusion. On the other experimental day, they were treated with a bolus infusion of dazoxiben (25 mg/kg in 50 ml of sterile saline) immediately before the endotoxin infusion and a maintenance infusion of dazoxiben (10 mg/kg per h in 50 ml of sterile saline/h) for 5.5 h after the endotoxin infusion. Dazoxiben alone at this dose produced no effect on eicosanoid, hemodynamic, or permeability measurements.

Arterial blood was drawn into liquid EDTA before and at 0.5, 1, 1.5, 2, 4, 5, and 6 h after the start of the endotoxin infusion. Leukocyte counts were determined from these samples with a Coulter counter (Coulter Electronics, Inc., Hialesh, FL).

Statistical analysis. Statistical significance was assumed for $P < 0.05$. The unpaired t test was used to determine differences resulting from the order of the experiments. Comparisons of differences between treated and untreated groups were made using analysis of variance (i.e., each animal served as its own control). Moreover, comparisons of base-line with experimental values within a group were made using analysis of variance. Multiple t tests were performed if the analysis of variance resulted in significance. Control and treated experiments were compared as were differences between base-line and experimental values at various times. This procedure may lead to concluding statistical significance due only to multiple sampling. That is, if samples are taken from the same population and if significance were assumed at $P < 0.05$, one would expect to find significance once per 20 tests. To eliminate the multiple sampling problem, we calculated the probability of the type error described above as follows. If P equals the probability of an event occurring (significance level) and q the probability of the event not occurring, then the probability of the event occurring k times in n trials is given by

$$P_n(k) = \frac{n!}{k!(n-k)!} P^k q^{n-k},$$

where $n!$ equals n factorial: $n! = n(n-1)(n-2) \ldots (2)(1)$.

As an example, if five calculations were made and significance found two times at $P < 0.05$, the probability of having found significance due to multiple sampling would be 2%. We accepted a probability of this type of error of 5%.

RESULTS

The order of the experiments did not produce any significant difference ($P > 0.05$, unpaired t test) in changes of measured parameters at times when their mean values were near maximum or minimum. That is, the animal's response to endotoxin 48 h after receiving endotoxin plus dazoxiben was unchanged from that seen when endotoxin was given first. Also, the response to endotoxin plus dazoxiben was unchanged regardless of order of experiment. These results are consistent with the work of Brigham et al. (3), who showed that the response of sheep to multiple injections of endotoxin was unchanged when 48 h elapsed between experiments. Therefore, we used each animal as its own control and all data were pooled.

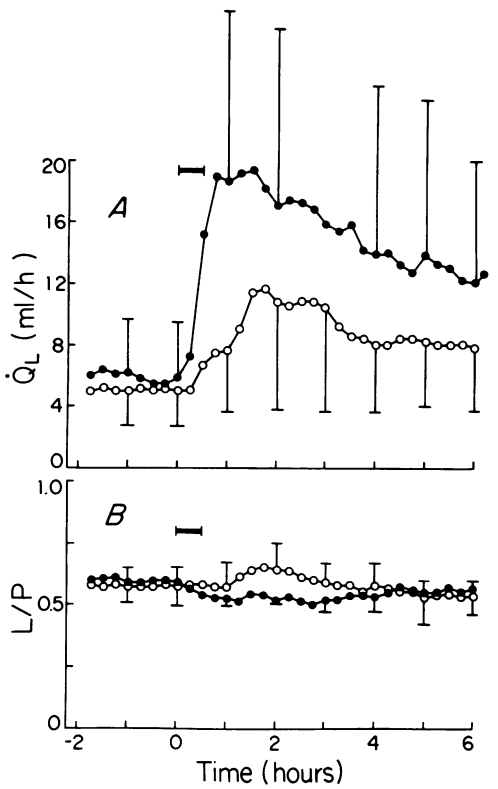

FIGURE 1 Effect of dazoxiben on $\dot{Q}_L$ after endotoxin infusion. $\dot{Q}_L$ (*A*) and L/P (*B*) were measured after infusion of endotoxin alone (filled circle) or endotoxin and dazoxiben (open circle). The infusion of endotoxin is shown by the bar. Values represent the means of six animals.

The peripheral leukocyte counts decreased rapidly after endotoxin was infused and remained low for the rest of the experiment. This decrease was seen in control as well as treated animals and the reduction was not statistically different between groups.

Average values of $\dot{Q}_L$ at each 15-min time period were plotted in Fig. 1 *A* for six control and six dazoxiben-treated experiments. Base-line $\dot{Q}_L$ averaged 5.87±3.22 ml/h before endotoxin infusion in control experiments and 5.08±2.23 ml/h before endotoxin in the dazoxiben-treated experiments. After the start of endotoxin infusion, control $\dot{Q}_L$ increased sharply, reaching a plateau at ~0.75 h, with a peak of 19.4±12.92 at 1.5 h, and then slowly decreased to 12.1±8.0, ml/h at 6 h. In treated animals $\dot{Q}_L$ increased less abruptly, reaching a lower peak of 11.7±8.3 ml/h at 1.75 h, and then declined slowly to 7.7±4.1 ml/h at 6 h. Each of the animals responded in a similar fashion. Changes in $\dot{Q}_L$ were significantly different in the two groups (analysis of variance). $\dot{Q}_L$ increased in all animals, whether or not they were pretreated with dazoxiben. However, the response in the treated

animals was statistically less than control at each measurement starting 15 min after the start of the infusion and lasting 2 h (paired t test). For both groups, $\dot{Q}_L$ was significantly greater than base line each hour after the infusion of endotoxin (paired t test).

Average values of total L/P were plotted in Fig. 1 *B*. In the control group, L/P showed the typical modest decrease during the 3–4-h hydrostatic phase (3) (significant by analysis of variance), then increased back to approximately the base-line level while $\dot{Q}_L$ was still elevated (permeability phase). By contrast, L/P in the animals treated with dazoxiben began to increase at the completion of the endotoxin infusion, reaching a peak at ~2 h, and then slowly decreased for the next 4 h back to the base-line value (significant by analysis of variance). Dazoxiben-treated animals did not show any clear indication of a hydrostatic phase of edema formation. L/P was significantly greater in treated experiments compared with control between 1.25 and 2.75 h after the start of endotoxin (paired t test).

Lung lymph clearance, calculated as the product of L/P and lymph flow, increased in both groups, Table I shows the average value of the 2-h base line plus values at each hour after infusion of endotoxin. When compared by paired t test, the control and treated groups were significantly different 1 h after infusion, but no difference was found at other times. Since significance was found at only one measurement, the error due to multiple sampling was too great to conclude that the clearance was different. The difference in clearance occurred when pulmonary pressures were markedly elevated in the untreated group (Fig. 2).

Pulmonary vascular hydrostatic pressures (arterial and wedge) are illustrated in Fig. 2. Base-line Ppa and Pw were 16.9±2.6 and 7.7±3.6 cm H_2O, respectively, for the control experiments. Endotoxin alone elicited a profound pulmonary arterial hypertension, reaching a mean peak value of 43.7±5.0 or 27 cm H_2O above bae line, coincident with the end of the infusion. Ppa slowly decreased from this value toward base line with a half-time of the order of 2–3 h for the remainder of the experiment. Ppa was significantly greater than base line each hour after the endotoxin (paired t test).

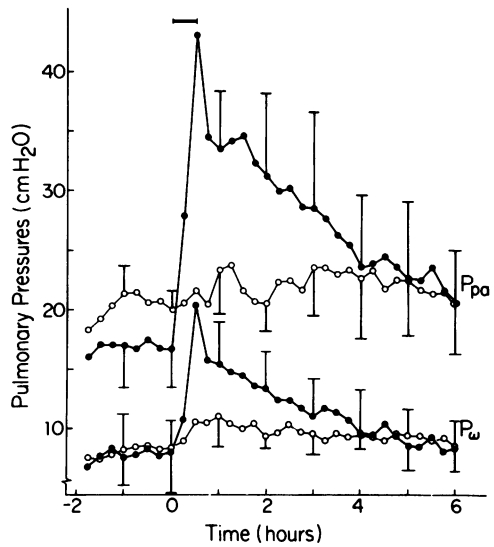

FIGURE 2 Effect of dazoxiben on pulmonary hemodynamic measurements after endotoxin infusion. Ppa and Pw were measured in animals receiving infusions of endotoxin alone (filled circle) or endotoxin and dazoxiben (open circle). The period of endotoxin is shown by the bar. Values represent the means of six animals.

Pw increased with approximately the same time course as Ppa, reaching a peak of 21.2±8.5 cm H_2O at 30 min, or ~14 cm H_2O above base line, and this elevation was significant for the first 3 h (paired t test). Ppa in treated animals increased only slightly and more gradually from a base line of 20.2±1.9 to a peak of 23.8±4.1 cm H_2O. The peak was just 4 cm H_2O above base line and occurred 45 min after the peak in the endotoxin-only experiments. This initial pressure elevation had essentially returned to base line by 2 h, but was followed by a second low broad rise of ~2–3 cm H_2O between 3 and 5 h. After dazoxiben, Pw was moderately increased from a base-line value of 8.1±2.5 to 11.0±2.7 cm H_2O 1 h after endotoxin. In treated animals, changes in Ppa were significant at 2 and again at 4 h. (paired t test).

Plasma TxA_2 concentration, as reflected by its stable

TABLE I

Average Values of Lung Lymph Clearance ($\dot{Q}_L \times L/P$) for Control and Dazoxiben-treated Goats

Treatment	Base line	Time after base line					
		1	2	3	4	5	6
				h			
Endotoxin only	3.40±1.56	9.76±5.76	8.92±6.00	7.76±6.00	7.12±4.68	7.48±5.00	6.64±4.16
Endotoxin plus dazoxiben	2.88±1.28	5.20±4.48	7.56±6.12	6.36±4.44	5.76±4.96	6.28±5.64	5.20±3.96

metabolite TxB_2, is graphed in Fig. 3 *A*. Endotoxin stimulated a rapid and profound synthesis and release of TxA_2, with TxB_2 reaching a peak of 13.1±11.3 ng/ml at 0.5 h. The initial rate of return toward base line after the end of the infusion was equally rapid. Dazoxiben treatment eliminated TxA_2 synthesis. Control animals' TxB_2 concentration was significantly greater than treated animals' at 30, 60, and 90 min, but not thereafter (paired *t* test). These increases in TxB_2 had a time response that roughly paralleled the pressure response seen in Fig. 2. Fig. 3 *B* shows the course of the average values of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) during the 8-h experiment. Peak values were 3.1±2.28 and 1.5±1.32 ng/ml in the treated and untreated animals, respectively. The levels of 6-keto-$PGF_{1\alpha}$ peaked earlier in the dazoxiben-treated group and were always greater than in the untreated group. $PGF_{2\alpha}$ concentration was elevated in both groups after endotoxin infusion. It was greater, although not significantly, in the treated group than control after endotoxin infusion. At 30 min after the infusion started, the dazoxiben-treated animals had an average $PGF_{2\alpha}$ concentration of 6.62±6.87 ng/ml, whereas in control experiments the concentration averaged 0.36±0.13 ng/ml. Elevation of $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ in the treated group over that of the untreated group was consistent with a shunting from TxA_2 into other pathways. It also demonstrated that the blocking action of dazoxiben on TxA_2 synthesis was quite specific and did not affect PG production adversely.

$\dot{Q}_t$, displayed in Fig. 4 *B*, decreased when only endotoxin was infused, from a base line of 4.1±0.3 to a nadir of 2.7±0.87 liters/min at 30 min. It recovered slightly, but remained depressed at ~3.3 liters/min for the next 3 h and then steadily increased, overshooting to 5.6±1.6 liters/min at the end of the experiment. In the dazoxiben-treated group $\dot{Q}_t$ decreased transiently from base line of 4.0±1.1 to 3.6±0.9 liters/min at 30 min. By 1 h it had returned to base line and remained at or above this value for the remainder of the experiment. $\dot{Q}_t$ in this group was significantly greater than in controls at every measurement between 0.5 and 2.5 h after the start of the infusion (paired *t* test). The minima of $\dot{Q}_t$ in both groups occurred at the time of peak pulmonary pressure, but the subsequent time course for these variables was markedly different.

Pa (Fig. 4 *A*) slowly but steadily decreased in the control group from a base-line value of 88.8±11.2 to ~67.8±18.6 torr at 6 h (significant by analysis of variance). After dazoxiben treatment, Pa decreased tran-

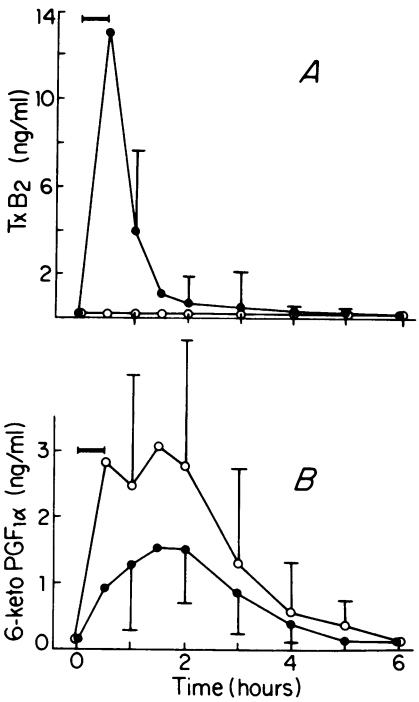

FIGURE 3 Effect of dazoxiben on plasma concentrations of TxB_2 (*A*) and 6-keto-$PGF_{1\alpha}$ (*B*) after endotoxin infusion. Plasma TxB_2 and 6-keto-$PGF_{1\alpha}$ levels were determined by radioimmunoassay after infusion of endotoxin alone (filled circle) or endotoxin and dazoxiben (open circle). The infusion of endotoxin is shown by the bar. Values represent the means of six animals.

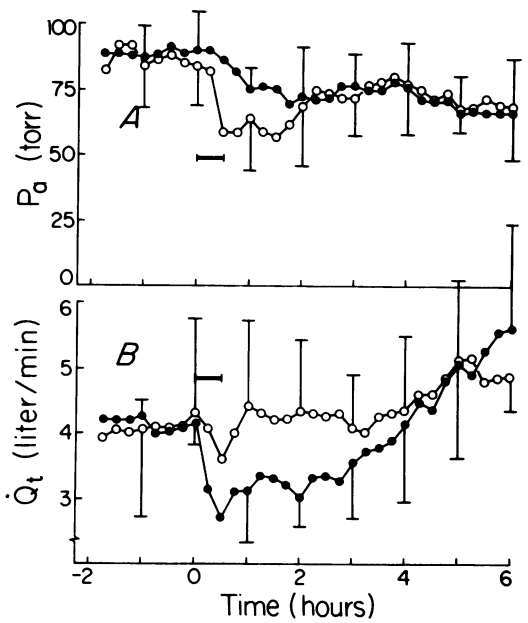

FIGURE 4 Effect of dazoxiben on systemic hemodynamic measurements after endotoxin infusion. Pa (*A*) and $\dot{Q}_t$ (*B*) were measured in animals receiving infusions of endotoxin alone (filled circle) or endotoxin and dazoxiben (open circle). The period of endotoxin infusion is shown by the bar. Values represent the means of six animals.

siently from 86.8±16.5 to 58.3±20.2 torr during the 1st h, then joined the control curve by 2.5 h, and remained at this level (significant by analysis of variance). Differences between the two groups were not statistically significant. The time courses of Pa did not seem to resemble those of other variables.

PVR and SVR are shown in Fig. 5 *A*, *B*. PVR for the most part paralleled the pattern demonstrated by Ppa. It increased rapidly from 2.3±0.8 to a peak of 8.6±4.2 cm H_2O/liter per min at 30 min, and then decreased fairly steadily toward base line. Dazoxiben prevented most of the changes in PVR. The control animals had a significantly higher PVR compared with dazoxiben-treated animals for 3.75 h after endotoxin (paired *t* test). SVR in the control group was elevated by ~50% of the base line during the latter half of the 30-min endotoxin infusion. It increased from 20.8±2.6 at base line to a peak of 31.6±9.3 torr/liter per min at 30 min, then returned to control levels after 1 h and remained approximately at that value for the next 3 h. A gradually augmenting vasodilatation then set in, with SVR falling to 11.7±2.7 torr/liter per min at 6 h. Dazoxiben treatment markedly altered the first 3 h: the early sudden vasoconstriction was replaced by a dilatation to a nadir of 11.5±6.3 torr/liter per min, then SVR joined the control curve for the final 3 h of this study.

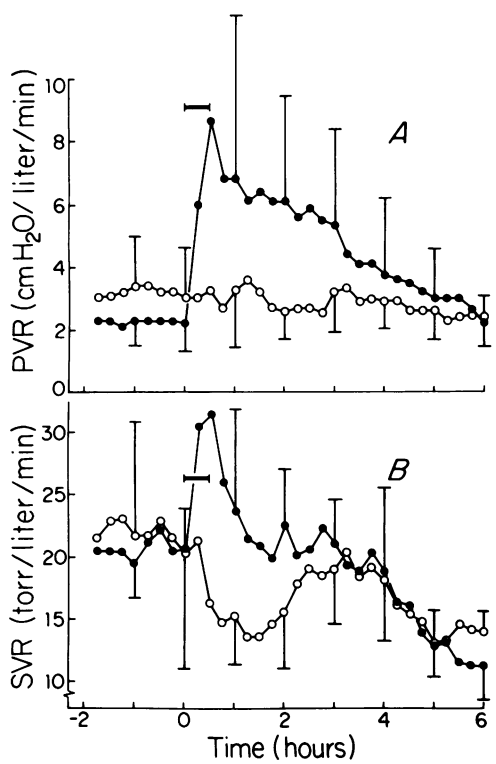

FIGURE 5 Effect of dazoxiben on PVR (*A*) and SVR (*B*) were measured in animals receiving endotoxin alone (filled circle) or endotoxin and dazoxiben (open circle). The period of endotoxin infusion is shown by the bar. Values represent the means of six animals.

DISCUSSION

These studies demonstrate that dazoxiben selectively inhibits TxA_2 synthesis after endotoxemia in goats. Increases in plasma levels of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ that were greater, although not significantly, in dazoxiben-treated than in control animals was consistent with shunting of endoperoxides into PGI_2 and $PGF_{2\alpha}$ synthesis. Whether such enhancement of PGI_2 synthesis is beneficial in endotoxemia is uncertain, although it has been suggested that it may play some protective role (7, 29).

Increased Pw in the control experiments most likely resulted from a general vasoconstriction throughout the lung. Left heart failure was ruled out, since left atrial pressure has been found to be unchanged or reduced by endotoxin infusion (3, 30). Inhibition of TxA_2 synthesis by dazoxiben essentially prevented increased Ppa, Pw, and PVR after endotoxin infusion, even though the vasoconstrictor $PGF_{2\alpha}$ concentration was elevated. Similar results following endotoxin infusion had been observed in sheep given a different thromboxane synthesis inhibitor (9).

As anticipated, lymph flow and L/P (Fig. 1 *A*, *B*) in the control endotoxin series of experiments were similar to those reported previously by others (3). The initial increases in $\dot{Q}_L$ have been referred to as the hydrostatic phase, since L/P fell and pulmonary vascular pressures rose as when left atrial pressure was increased (31). However, one could not exclude the possibility that permeability was also increased during this period, and that part of the elevated $\dot{Q}_L$ was the result of that increase. Early permeability changes were confirmed when most of the pulmonary pressure increase mediated by TxA_2 was blocked by treating with dazoxiben (Fig. 2) and only about half the increased $\dot{Q}_L$ was eliminated. Since L/P was also elevated, these results could be explained by either a modest increase in permeability (P), or by a large increase of surface area (S) together with a reduced flux per unit area (to explain the rise of L/P). This would require some reduction in microvascular pressure, of which no indication could be found in the dazoxiben-treated animals (Fig. 2). There was also no indication that S had increased as by capillary recruitment, since PVR was unchanged. Therefore, increased $\dot{Q}_L$ after dazoxiben injection and endotoxin infusion most likely was the result of increased permeability. The increased lymph clearance in the dazoxiben-treated animals was

also most likely due only to increased permeability. However, lymph clearance also increases as a result of increased hydrostatic pressure and differences between groups at 1 h can be explained by the increased pressure. The permeability alteration or injury starts within 15 min of the onset of endotoxin. Since $\dot{Q}_L$ and L/P reached a peak at ~1 h after the end of infusion, it appeared that the injury was transient and began to reverse at that time.

The reduction in $\dot{Q}_L$ in the dazoxiben-treated group relative to control may have been the result of some protection against changes in permeability by the dazoxiben. However, it was more likely simply due to the strikingly lower pulmonary microvascular pressure (Fig. 2). In other words, the same degree of permeability change may have existed in the two groups, but in the control series the higher microvascular pressure resulted in more filtration of relatively protein-poor fluid. Surface area of the fluid exchanging regions may also have increased in either group leading to an increase in $\dot{Q}_L$, but as discussed above it is unlikely to have changed after dazoxiben treatment. Thus, TxA_2 mediates part of the pulmonary hypertension after endotoxin infusion, since pretreatment with an inhibitor of TxA_2 synthesis can prevent increases in Ppa and Pw. It may not be the only vasoconstrictor present during endotoxemia, since the vasodilator PGI_2 levels were elevated in treated animals. That is, the increased vasodilation with dazoxiben may have reduced Ppa resulting from either vasoconstrictors. TxA_2, however, plays no role in the permeability changes in this disease model.

$\dot{Q}_t$ was sharply reduced when endotoxin was given alone (Fig. 4 *B*). This could have resulted from a decreased return to the left heart, resulting from the increased PVR, or decreased myocardial contractility. Ppa and PVR increased in concert with the initial drop in $\dot{Q}_t$, but Ppa then steadily decreased until the end of the experiment, whereas $\dot{Q}_t$ remained depressed and fairly stable for about 3 h before starting to increase toward base line. Finally, $\dot{Q}_t$ at 6 h was about one-third higher than base-line levels, while Ppa remained elevated. Thus, the course of $\dot{Q}_t$ does not appear to be readily explainable in terms of venous return to the left heart.

Lefer et al. (32) have shown that a synthetic analogue of TxA_2 is a potent coronary artery vasoconstrictor that can cause myocardial ischemia and death in rabbits. These effects can be blocked by a thromboxane receptor antagonist, implicating TxA_2 as a potent coronary vasoconstrictor. Therefore, it is possible and perhaps probable that myocardial function was depressed and the heart was unable to maintain normal $\dot{Q}_t$. Pretreatment in our goats with dazoxiben prevented almost all of the fall in $\dot{Q}_t$, perhaps by preventing myocardial depression by preventing coronary artery constriction and allowing better perfusion to the heart.

We conclude that pretreatment coupled with a continuous infusion of dazoxiben during endotoxemia prevented the synthesis of TxA_2, but not PGI_2 or $PGF_{2\alpha}$. The prevention of TxA_2 synthesis did not prevent endotoxin-induced permeability changes. In fact, by eliminating the hypertensive phase, it reveals that the permeability change occurs soon after the start of the endotoxin infusion. The pulmonary arterial and pulmonary wedge hypertension resulting from endotoxin was the result of vasoconstriction mediated by TxA_2. This vasoconstriction occurred throughout the pulmonary vascular bed, since Pw was also elevated, but others have shown no elevation in left atrial pressure. Impaired $\dot{Q}_t$ was also mediated by the TxA_2, possibly as a result of coronary artery constriction and a resultant myocardial depression. Since TxA_2 was produced in the lung (8, 9) and its plasma half-life was only seconds, it was probably rapidly converted to the weak vasoconstrictor TxB_2 before reaching the vasoconstrictor sites in the systemic circulation. Therefore, systemic vasoconstriction was not sustained.

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