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Effect of nafazatrom and indomethacin on pulmonary removal of prostaglandin E_1 after endotoxin in rabbits

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1 We compared the effects of endotoxin on pulmonary prostaglandin E_1 (PGE_1) removal in groups of rabbits pretreated with the cyclo-oxygenase inhibitor, indomethacin, or nafazatrom (Bay g 6575), which has been shown to increase plasma prostacyclin concentrations.

2 In untreated animals, endotoxin transiently decreased pulmonary removal of [^{3}H]-PGE_1, caused pulmonary hypertension, systemic hypotension and increased plasma concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$.

3 Indomethacin pretreatment prevented the transient decrease in pulmonary removal of [^{3}H]-PGE_1 in response to endotoxin, prevented the haemodynamic effects and inhibited prostaglandin synthesis. Pretreatment with nafazatrom did not affect the decreased pulmonary removal of [^{3}H]-PGE_1, exacerbated the haemodynamic response, reduced survival and potentiated the increase in circulating 6-keto-$PGF_{1\alpha}$.

4 We conclude that indomethacin acts to prevent the depression of pulmonary [^{3}H]-PGE_1 removal by eliminating surface area changes associated with endotoxin-induced pulmonary vasoconstriction.

5 These data suggest that nafazatrom treatment results in exacerbation of the endotoxin-induced systemic hypotension presumably due to its effect on increased plasma prostacyclin during the later phase of endotoxaemia.

Introduction

Escherichia coli endotoxin infusion causes acute lung injury manifested in part by pulmonary hypertension, systemic hypotension, activation of the arachidonic acid cascade, and increased lung microvascular permeability (Brigham *et al.*, 1979; Bult *et al.*, 1980; Demling *et al.*, 1981). Histological evidence shows pulmonary leukocyte sequestration and endothelial cell damage (Meyrick & Brigham, 1982). In addition to neutrophils (Heflin & Brigham, 1982), prominent mediating roles for the complement system (Bult *et al.*, 1985) as well as direct endothelial cell injury (Brigham & Meyrick, 1986) have been reported.

Acute lung injury of various aetiologies is known to impair metabolic function of this organ, including removal of biogenic amines (Block & Fisher, 1977; Block & Schoen, 1981; Dobuler *et al.*, 1982) and hydrolysis of substrates of angiotensin-converting enzyme (Catravas *et al.*, 1983). Removal of prostaglandins of the E series is also depressed by lung injury induced by α-naphthylthiourea (Bakhle, 1982), prolonged cardiopulmonary bypass (Pitt *et al.*, 1982) and hyperoxia (Klein *et al.*, 1978). Since the lung is an important site for inactivation of prostaglandins of the E and F series and may also be an important site of biosynthesis of these prostaglandins as well as prostacyclin (Gryglewski *et al.*, 1978), we examined the pulmonary removal of [^{3}H]-prostaglandin E_1 ([^{3}H]-PGE_1) by the anaesthetized rabbit after endotoxin administration to determine if (1) endotoxin impairs pulmonary PGE_1 removal, (2) this decrease in removal is dependent on simultaneous production of cyclo-oxygenase products (i.e. saturation of the uptake process), and (3) whether a drug proposed to increase plasma prostacyclin levels, and thereby perhaps attenuate microvascular injury (Fletcher & Ramwell, 1980; Krausz *et al.*, 1981), can modify the pulmonary response to endotoxin. To examine the effect of arachidonic acid metabolites produced during endotoxaemia on pulmonary metabolic and hemodynamic variables we used either the cyclo-oxygenase inhibitor, indomethacin, or the putative antithrombotic drug, nafazatrom (Seuter *et al.*, 1979), previously

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postulated to increase prostacyclin levels (Vermylen *et al.*, 1979; Deckmyn *et al.*, 1983; Fischer *et al.*, 1983).

Methods

Animal preparation

New Zealand White male rabbits (3.5 to 4.0 kg) were anaesthetized with a mixture of allobarbitone (50 mg ml^{-1}) and urethane (200 mg ml^{-1}) administered intravenously. A tracheostomy was performed and the animals were ventilated with a gas mixture of O_2 and N_2 ($FiO_2 = 0.5$). Arterial blood gases were measured periodically throughout the experiment (Radiometer BMS3, Copenhagen). Tygon catheters were introduced into the right atrium and the carotid artery for radioisotope injection and blood sampling, respectively. A catheter was advanced into the pulmonary artery via the jugular vein, with placement being determined by the pressure tracing, and another catheter was placed in the descending aorta via a femoral artery. These catheters were connected to Statham P23a pressure transducers, the outputs of which were continuously recorded with a Grass oscillographic recorder (Model 7C). All animals received a constant infusion (0.5 ml min^{-1}) of dextrose/dextran solution (NaCl 9.0 g l^{-1}; $NaHCO_2$ 4.0 g l^{-1}; dextrose 50 g l^{-1}; 70,000 MW dextran 30 g l^{-1}) throughout the experiment to compensate for blood loss due to repeated sampling. The total volume infused was between 100 and 150 ml over 3 to 4 h.

Experimental procedure

After a stabilization period of at least 30 min following surgical preparation, baseline measurements of [^{3}H]-PGE_1 removal were made by double indicator dilution techniques (see below). At the same time, blood samples from the carotid artery were collected into tubes treated with heparin and indomethacin for determination of PGE_2 and 6-keto-$PGF_{1\alpha}$ levels by radioimmunoassay.

E. coli endotoxin (Lipopolysaccharide B 055:B5 obtained from Difco Laboratories, Detroit, Michigan) was administered at a dose of 1 mg kg^{-1} as a slow intravenous infusion (1 ml saline) over approximately 1 to 2 min. Metabolic and haemodynamic measurements were repeated at the peak of the pulmonary hypertensive response, normally 20 to 25 min after endotoxin administration, and also at 90 and 150 min after endotoxin.

Animals were divided into six groups as shown in Table 1. Control animals (Group A) received no interventions. Endotoxin-treated animals (Group B) received endotoxin 1 mg kg^{-1} as described above.

Table 1 Experimental groups and treatments

Group A ($n = 8$) Control, no interventions.
Group B ($n = 9$) Endotoxin (1 mg kg^{-1}).
Group C ($n = 12$) Nafazatrom pretreated (1 mg kg^{-1}, i.v.) 20 min before endotoxin.
Group D ($n = 6$) Indomethacin pretreated (10 mg kg^{-1}, i.v.) 20 min before endotoxin.
Group E ($n = 6$) Nafazatrom control (1 mg kg^{-1}), no endotoxin.
Group F ($n = 3$) Indomethacin (10 mg kg^{-1}) 20 min before nafazatrom (1 mg kg^{-1}) 20 min before endotoxin.

Nafazatrom-pretreated animals (Group C) received nafazatrom at a dose of 1 mg kg^{-1} (1 ml saline with pH adjusted to 12 with NaOH) as an intravenous bolus 20 min before endotoxin. Indomethacin-pretreated animals (Group D) received indomethacin 10 mg kg^{-1} (in 5 ml 0.1 N sodium carbonate) 20 min before endotoxin as an intravenous infusion over 2 min. Nafazatrom control animals (Group E) received nafazatrom 1 mg kg^{-1} only, to serve as a drug treatment control. An additional control group was treated with indomethacin, nafazatrom and endotoxin (Group F). These animals were pretreated with both indomethacin (40 min before) and nafazatrom (20 min before) prior to endotoxin.

Prostaglandin radioimmunoassay

Blood samples for the radioimmunoassays were obtained simultaneously from the pulmonary artery and the carotid artery by drawing 2 ml blood into iced syringes pretreated with heparin and indomethacin to prevent *ex vivo* prostaglandin synthesis. The syringes were immediately placed in ice. Blood samples were then transferred into polypropylene tubes and centrifuged to separate the plasma which was removed and frozen until assayed.

Radioimmunoassay reagents for PGE_2 and 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, were obtained from New England Nuclear (Boston, MA.). The tracer isotope was ^{125}I. Plama samples were assayed directly after an appropriate dilution (between 2 and 10 fold) with buffer solution containing 0.9% NaCl, 0.01 M EDTA, 0.3% bovine gamma-globulin, 0.005% Triton X–100 and 0.05% sodium azide in 50 mM phosphate buffer, pH = 6.8. All samples were assayed in duplicate and the average value reported. To account for possible non-specific binding effects in plasma samples, a volume of prostaglandin-free plasma equal to the volume of plasma in each unknown tube was added to each of the eight standard curve samples. Additionally, as a method blank, a sample of prostaglandin-free plasma was treated as an

unknown sample. This blank was typically > 95% B/Bo.

For the PGE_2 assay the antiserum exhibits the following cross-reactivity at 50% B/Bo: PGE_1 – 3.7%; dihydro-keto PGE_2 – 0.4%; PGA_2 – 0.4%; $PGF_{1\alpha}$ – 0.03%; TxB_2 – 0.02%; all other compounds tested – < 0.01%. The sensitivity of the assay was 4 pg ml^{-1}. For the 6-keto-$PGF_{1\alpha}$ assay the antiserum exhibits the following cross-reactivities at 50% B/Bo: PGF_2 – 2.6%; PGE_1 – 1.9%; TxB_2 – 1.4%; PGE_2 – 1.1%; $PGF_{1\alpha}$ – 0.8%; PGA_1 – 0.2%; PGD_2 – 0.2%; PGA_2 – 0.04%; all other compounds tested – < 0.01%. The sensitivity of the assay was 0.2 ng ml^{-1}.

Indicator dilution measurement of [^{3}H]-PGE_1 removal

Measurement of [^{3}H]-PGE_1 removal by rabbit lungs using multiple indicator dilution techniques has previously been described in detail (Pitt *et al.*, 1983). Briefly, a 0.9 ml bolus injection containing trace quantities of [^{3}H]-PGE_1 (3.8 μCi, 0.06 nmol) and [^{14}C]-dextran (0.6 μCi, 0.5 mg, the intravascular marker), was made into the right atrium while blood was simultaneously collected from the carotid artery into a fraction collector for 20 s at a rate of 20 ml min^{-1} and 1 s per sample. Blood was collected into test tubes containing 2 ml of heparinized saline and then centrifuged. Total radioactivity was measured in a 0.5 ml aliquot of supernatant by liquid scintillation spectrometry. For determination of [^{3}H]-PGE_1 metabolites, another 0.5 ml aliquot was extracted into acidified ethyl acetate, evaporated to dryness, and resuspended in 0.1 ml of ethyl acetate. This sample was then applied to a silica gel column which separated the major metabolites of [^{3}H]-PGE_1 in a single step (Altiere *et al.*, 1981).

Fractional concentrations of each isotope in every sample collected were calculated as the measured concentration divided by the total amount injected. Instantaneous percentage removal of [^{3}H]-PGE_1 was calculated as:

$$\%R(PGE) = (FC_{DEX} - FC_{PGE}) / FC_{DEX} \times 100$$

where %R(PGE) = percentage removal of [^{3}H]-PGE_1; FC_{DEX} = fractional concentration of [^{14}C]-dextran; and FC_{PGE} = fractional concentration of [^{3}H]-PGE_1 corrected for any metabolite present in the sample. Cardiac output, volume of distribution and mean transit time were also calculated from the [^{14}C]-dextran arterial outflow curve (Meir & Zierler, 1954). For comparative purposes, R([^{3}H]-PGE_1) is reported at the peak of the dextran reference outflow curve.

Statistical analysis

Data are given as the mean and s.e.mean. Comparisons within each group were made with a one-way analysis of variance, followed by Dunnett's test comparing each group mean to the baseline mean values for the group (Zar, 1984). Comparisons between the nafazatrom-pretreated endotoxin group and the endotoxin-treated group were made by an unpaired Student's t test. We accepted $P < 0.05$ as indicating statistical significance.

Results

Haemodynamic Response

Mean haemodynamic data for Groups A – D are shown in Figure 1. There was no significant change in pulmonary arterial pressure, systemic arterial pressure or cardiac output during the experiment in the control group (Group A). Endotoxin treatment (Group B) caused a pulmonary hypertensive response which began about 15 min after endotoxin infusion and peaked about 20 to 25 min later. Concurrent wtih the pulmonary hypertension was a fall in mean systemic arterial pressure and a decrease in cardiac output. Systemic arterial pressure remained depressed for the duration of the experiment, whereas cardiac output and pulmonary arterial pressure returned to control values by 90 and 150 min, respectively.

When rabbits were treated wtih nafazatrom before endotoxin administration (Group C), the haemodynamic response was more pronounced, resulting in a significantly lower systemic arterial pressure than with endotoxin alone. Nafazatron pretreatment also caused a dramatic decrease in the survival of endotoxin-treated animals, apparently the result of circulatory collapse. In the endotoxin group (Group B) all 9 animals survived the experiment. With nafazatrom pretreatment (Group C), survival fell to 7/12 at 90 min and only 6 animals survived for 150 min. Indomethacin pretreatment (Group D) completely prevented the haemodynamic alterations due to endotoxin, and all of the indomethacin pretreated animals survived the experiment.

As shown in Table 2, nafazatrom alone, given to a control group of animals (Group E), had no effect on blood pressures or cardiac output. Table 2 also shows that indomethacin prevented the haemodynamic alterations when both nafazatrom and endotoxin were present (Group F).

Prostaglandin synthesis

Figure 2 shows the aortic plasma concentrations of PGE_2 and the stable hydrolysis product of PGI_2, 6-keto-$PGF_{1\alpha}$, in Groups A – D. Endotoxin administration (Group B) significantly increased PGE_2 concentrations by 90 min; this increase was maintained at 150 min. Prostacyclin levels were also increased after endotoxin administration. Aortic plasma concentra-

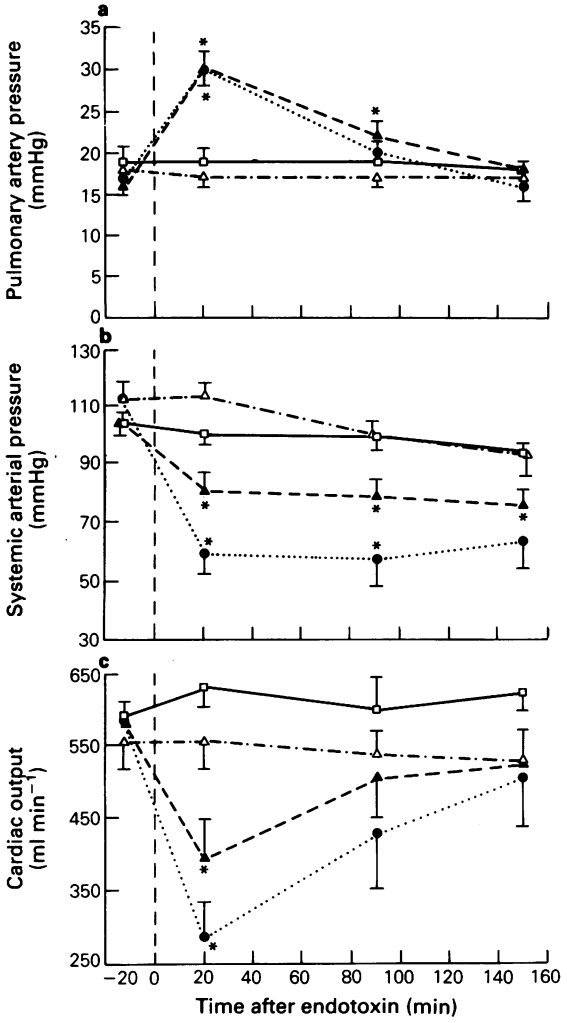

Figure 1 (a) Pulmonary arterial pressure in the control (□), endotoxin (▲), nafazatrom pretreated endotoxin (●), and indomethacin pretreated endotoxin (△) groups of rabbits. Endotoxin (1 mg kg^{-1}) was given at time zero. Indomethacin (10 mg kg^{-1}) or nafazatrom (1 mg kg^{-1} was given 20 min before endotoxin. Values shown are mean with s.e.mean shown by vertical lines; some s.e.mean measurements are omitted for clarity. * $P<0.05$ compared to baseline. (b) Systemic arterial pressure in the same four groups of animals. (c) Cardiac output as determined by the arterial reference outflow curve in the same four groups.

tions of 6-keto-PGF$_{1\alpha}$ were significantly increased at 90 and 150 min after endotoxin.

Rabbits pretreated with nafazatrom (Group C) also showed significantly increased plasma concentrations of both PGE_2 and 6-keto-PGF$_{1\alpha}$ after endotoxin. Aortic PGE_2 levels were significantly higher than baseline values at 90 and 150 min after endotoxin, but due to the large variability between animals were not significantly different from endotoxin-treated animals (Group B). Nafazatrom pretreatment did however result in a significant elevation of plasma 6-keto-PGF$_{1\alpha}$ compared to endotoxin treatment alone. In contrast, indomethacin pretreatment (Group D) completely prevented increased synthesis of both PGE_2 and 6-keto PGF$_{1\alpha}$ after endotoxin and reduced plasma concentrations to very low levels.

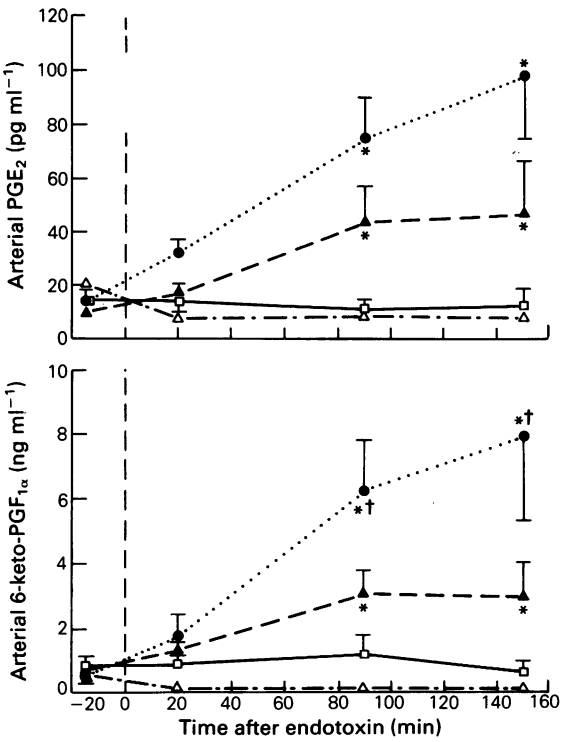

Figure 2 (a) Arterial plasma concentration of prostaglandin E_2 (PGE_2) in control (□), endotoxin (▲), nafazatrom pretreated endotoxin (●) and indomethacin pretreated endotoxin (△) groups of rabbits. Values shown are mean with s.e.mean shown by vertical lines. *$P<0.05$ compared to baseline values; † significantly different from endotoxin treatment alone. (b) Arterial plasma concentration of 6-keto-PGF$_{1\alpha}$ in the same four groups.

Table 2 Effect of nafazatrom alone and effect of indomethacin on nafazatrom plus endotoxin on haemodynamics, aortic prostaglandin levels, and pulmonary prostaglandin E_1 (PGE_1) removal in the anaesthetized rabbit

Exp. Group	*Time* (min)	*Ppa* (mmHg)	*Pa* (mmHg)	CO (ml min^{-1})	PGE_2 (pg ml^{-1})	*6-keto* $PGF_{1\alpha}$ (ng ml^{-1})	PGE_1 *removal* (%)
Group E Nafazatrom Control ($n = 6$)	Baseline	17 ± 1	112 ± 7	590 ± 36	17.7 ± 3.3	1.3 ± 0.6	82.7 ± 3.7
	+ 20	18 ± 1	109 ± 7	554 ± 36	15.4 ± 2.1	0.8 ± 0.2	82.7 ± 3.9
	+ 90	18 ± 2	105 ± 8	545 ± 58	13.5 ± 1.3	0.7 ± 0.1	81.2 ± 3.7
	+ 150	16 ± 1	104 ± 8	564 ± 33	11.8 ± 1.5	1.1 ± 0.6	86.4 ± 2.3
Group F Indo/Nafaz Endotoxin ($n = 3$)	Baseline	14 ± 1	108 ± 4	552 ± 17	21.2 ± 6.7	0.2 ± 0.1	85.0 ± 0.9
	+ 20	15 ± 1	111 ± 6	595 ± 33	6.0 ± 1.6	0.1 ± 0.0	88.3 ± 0.9
	+ 90	13 ± 1	105 ± 3	553 ± 51	6.8 ± 1.9	0.1 ± 0.0	90.2 ± 0.3
	+ 150	13 ± 1	98 ± 4	532 ± 36	12.0 ± 5.3	0.1 ± 0.0	90.5 ± 0.8

Values shown are mean + s.e.mean. Ppa = pulmonary artery pressure; Pa = systemic arterial pressure; CO = cardiac output. Group E received 1 mg kg^{-1} nafazatrom i.v. only. Group F received 10 mg kg^{-1} indomethacin, 40 min before then 1 mg kg^{-1} nafazatrom, 20 min before endotoxin.

In control experiments nafazatrom treatment by itself (Group E) did not affect plasma levels of either PGE_2 or 6-keto-$PGF_{1\alpha}$ (Table 2). Also, the stimulatory effect of nafazatrom on endotoxin-induced arachidonic acid metabolism was completely prevented by indomethacin pretreatment (Group F).

Pulmonary metabolic response

Pulmonary removal of [^{3}H]-PGE_1 in Groups A – D, measured by double indicator dilution techniques, is shown in Figure 3. Under control conditions (Group A), single-pass pulmonary removal of [^{3}H]-PGE_1 was 85 to 90% and remained unchanged throughout the experiment. After endotoxin administration (Group B), [^{3}H]-PGE_1 removal fell significantly from 88% to 73% by 20 min, but returned to baseline values within 1 h. Nafazatrom pretreatment (Group C) did not affect the transient endotoxin-induced decrease in pulmonary [^{3}H]-PGE_1 removal, while indomethacin (Group D) completely abolished this response.

Table 2 shows that nafazatrom treatment, by itself, had no effect on pulmonary [^{3}H]-PGE_1 removal (Group E). Furthermore, indomethacin also prevented the depression of [^{3}H]-PGE_1 removal in animals that received nafazatrom plus endotoxin (Group F).

Figure 4 shows the mean pulmonary transit time for [^{14}C]-dextran in Groups A – D. There was a significant increase in transit time, associated with the concomitant decrease in cardiac output, in both the endotoxin treated and the nafazatrom-pretreated endotoxin group that returned to baseline values by 150 min. Figure 5 shows the changes in the calculated blood volume of distribution (pulmonary blood volume) for [^{14}C]-dextran in the same four groups. There was also a significant transient decrease in volume of distribution after endotoxin in the endotoxin-treated and nafazatrom pretreated groups.

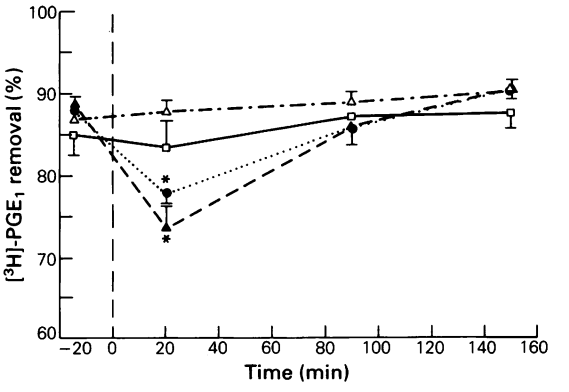

Figure 3 Pulmonary removal of [^{3}H]-prostaglandin E_1 ([^{3}H]-PGE_1) determined by multiple indicator dilution technique in control (□), endotoxin (▲), nafazatrom pretreated endotoxin (●) and indomethacin pretreated endotoxin (△) groups of rabbits. Results shown are the mean for the instantaneous removal at the peak of the reference curve; vertical lines show s.e.mean. * Significantly different from baseline value.

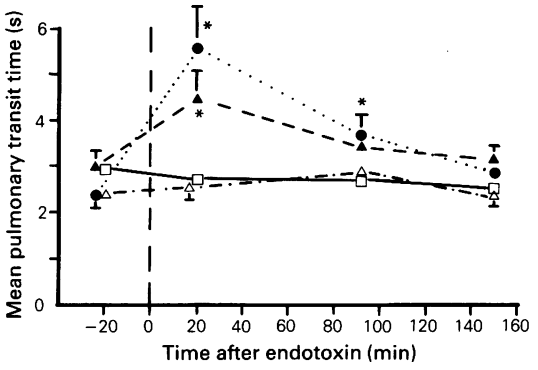

Figure 4 Mean pulmonary transit time for [^{14}C]-dextran in the control (□), endotoxin (▲), nafazatrom-treated endotoxin (●), and indomethacin pretreated endotoxin (△) groups of rabbits. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ compared to baseline value.

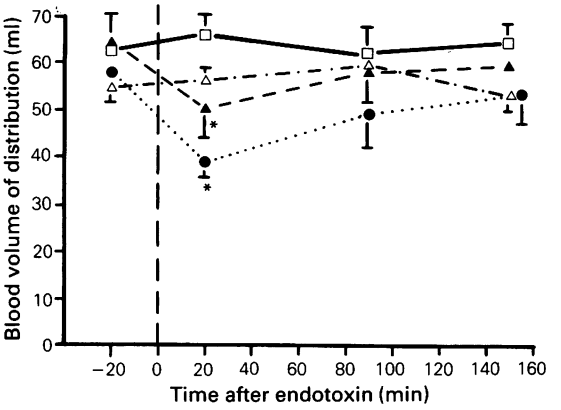

Figure 5 Mean blood volume of distribution for [^{14}C]-dextran in the control (□), endotoxin (▲), nafazatrom-treated endotoxin (●), and indomethacin pretreated endotoxin (△) groups of rabbits. Values shown are mean with s.e.mean shown by vertical lines. * $P < 0.05$.

Discussion

Haemodynamic response

Metabolites of arachidonic acid play a key role in both the transient pulmonary hypertension and the prolonged systemic hypotension after endotoxin (Brigham *et al.*, 1979; Bult *et al.*, 1980; Brigham & Meyrick, 1986). Transient production of thromboxane A_2 is thought to mediate the acute pulmonary hypertensive phase in sheep (Ogletree & Brigham, 1982), and Bult and colleagues (1980) have postulated that increased synthesis of prostacyclin accounts for the observed systemic hypotension after endotoxin administration in rabbits.

In the experiments decribed here, indomethacin prevented both the pulmonary hypertension and the systemic hypotension after endotoxin (Figure 1), supporting the concept that one or more vasoconstrictor products of cyclo-oxygenase are responsible for the endotoxin-induced pulmonary hypertension and that prostacyclin is a mediator of endotoxin-induced systemic hypotension. Furthermore, we have also demonstrated increased synthesis of PGE_2 (Figure 2), which also may contribute to the hypotension associated with endotoxaemia.

The later, prolonged systemic hypotensive response to endotoxin was exacerbated by nafazatrom, probably as a result of increased plasma concentrations of the potent vasodilator, PGI_2. This interaction of nafazatrom with endotoxin must be due to alterations in arachidonic acid metabolism rather than some other action of nafazatrom since indomethacin pretreatment completely prevented the synergistic effect of nafazatrom and endotoxin (Group F, Table 2).

Action of nafazatrom

Nafazatrom has previously been shown to be effective in reducing thrombus formation after experimental arterial damage (Seuter *et al.*, 1979). It has been suggested that an increased plasma concentration of prostacyclin is responsible for the drug's effect *in vivo* (Vermylen *et al.*, 1979). Although the mechanism of action of the drug *in vivo* is not well understood, nafazatrom has been shown to stimulate PGI_2 synthesis *in vitro* by acting as a substrate for the peroxidase-catalyzed reduction of hydroperoxy fatty acids that are irreversible inactivators of PGI_2 synthase (Marnett *et al.*, 1984) as well as acting as an inhibitor of the 15-hydroxy-prostaglandin dehydrogenase which is responsible for the degradation of prostaglandins (Wong *et al.*, 1982). The present experiments show that nafazatrom treatment *per se* did not affect plasma concentrations of PGE_2 and PGI_2 (Table 2). However, when the arachidonic acid cascade was stimulated by endotoxin infusion, nafazatrom significantly increased the concentration of PGI_2 (Figure 2). Whether this phenomenon is due to increased synthesis of PGI_2 or impaired degradation is not known.

Prostaglandin E_1 removal

Elevated plasma levels of prostaglandins after endotoxaemia may be due to increased synthesis,

decreased degradation, or both. Spannhake and co-workers (1983) found evidence suggesting increased cyclo-oxygenase activity after endotoxin in the dog. Pulmonary removal of E and F series prostaglandins consists of a specific carrier-mediated transport process followed by intracellular degradation by 15-hydroxy prostaglandin dehydrogenase (PGDH) and prostaglandin reductase (Anderson & Eling, 1976; Bito *et al.*, 1977). Although inhibitors of the transport process impair pulmonary removal of PGE_1 (Pitt *et al.*, 1983; Dawson *et al.*, 1984), inhibitors of PGDH which prevent pulmonary inactivation of PGE_1 do so by inhibition of prostaglandin uptake rather than inhibition of PGDH (Bakhle, 1979). Therefore, although Nakano & Prancan (1973) found that lung homogenates from endotoxin-treated rats had an impaired enzymatic degradation of PGE_1, the functional significance of this finding *in vivo* is not clear.

Previous reports from this laboratory have characterized the extensive removal and metabolism of [^{3}H]-PGE_1 during a single passage through the lung (Pitt *et al.*, 1983). Indeed, changes in the ability of the lung to metabolize many substances is thought to reflect the functional integrity of the pulmonary microvascular endothelium (Gillis & Catravas, 1982). Results of the present study indicate that pulmonary removal of [^{3}H]-PGE_1 was transiently reduced after endotoxin administration, but returned to normal within 1 h. Several factors other than the metabolic integrity of the lung may affect the measured removal of [^{3}H]-PGE_1 in an *in vivo* model where haemodynamic variables cannot be held constant. Among these are changes in perfused vascular surface area and transit time through the lung (Gillis, 1986). Of these two factors, we found that mean transit time increased significantly during the acute hypertensive response in both the endotoxin and the nafazatrom-treated endotoxin group (Figure 4). Increased mean transit time *per se* would tend to increase removal of [^{3}H]-PGE_1 due to increased time for substrate-carrier interaction, and therefore to oppose the observed fall in removal seen after endotoxin.

It is also likely that perfused vascular surface area was altered during the pulmonary hypertensive response, due to pulmonary vasoconstriction and decreases in cardiac output. The net effect of these responses on capillary recruitment or derecruitment will depend in part on the site of vasoconstriction (arterioles or venules). However, the decreased volume of distribution for the reference indicator in the endotoxin-treated animals (Figure 5) strongly suggests a decreased pulmonary blood volume and a reduced capillary surface area. Thus, a reduced vascular surface area would explain the decreased pulmonary [^{3}H]-PGE_1 removal due to a loss in available uptake sites. This hypothesis would also explain the ability of indomethacin to inhibit the transient depression in pulmonary [^{3}H]-PGE_1 removal. Inhibition of endotoxin-induced haemodynamic changes would also presumably eliminate major changes in perfused vascular surface area, and therefore prevent the decrease in pulmonary [^{3}H]-PGE_1 removal. The transient decrease in [^{3}H]-PGE_1 removal does not however explain the increased plasma prostaglandin levels in these experiments since [^{3}H]-PGE_1 removal was normal during the later phase when prostaglandin levels were elevated.

In conclusion, we have shown that endotoxin caused a transient decrease in pulmonary [^{3}H]-PGE_1 removal which is apparently not due to saturation of the removal process by increased endogenous prostaglandin levels since [^{3}H]-PGE_1 removal was normal at 90 and 150 min after endotoxin, despite increased plasma prostaglandin levels. Indomethacin may act to prevent the decrease in removal of [^{3}H]-PGE_1 after endotoxin by preventing decreases in perfused vascular surface area by preventing production of vasoconstrictor cyclo-oxygenase products. Nafazatrom, due to its ability to elevate plasma prostacyclin levels during the later phase of endotoxaemia, significantly exacerbated the systemic hypotension and decreased survival, and had no protective effect on the early pulmonary hypertension or decreased pulmonary removal of [^{3}H]-PGE_1.

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