

Roles of Platelet-Activating Factor and Thromboxane in Group B Streptococcus-Induced Pulmonary Hypertension in Piglets

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ABSTRACT. Platelet-activating factor causes pulmonary hypertension, shock, hypoxemia, neutropenia, and increased pulmonary vascular permeability; some of its effects are due to thromboxane A₂ release. Evidence for a possible role of these mediators in the genesis of group B Streptococcus (GBS)-induced pulmonary hypertension was sought using specific receptor antagonists for PAF and thromboxane A₂ (TxA₂) in anesthetized, ventilated piglets (≤ 12 d of age; $n = 22$). Infusion of 1×10^8 GBS/kg/min for one hour resulted in a sustained and significant increase in pulmonary artery pressure (P_{PA}) from 17 ± 1 to 35 ± 3 torr. Pretreatment with the TxA₂ antagonist SQ 29548 (0.75 mg/kg intravenous), completely inhibited the effect of GBS on P_{PA} . Pretreatment with either platelet-activating factor antagonists SRI 63072 (3 mg/kg intravenous) or SRI 63441 (1 mg/kg) did not affect the pulmonary hypertension due to GBS infusion. GBS-induced pulmonary hypertension could be reversed by SQ 29548; SRI 63072 did not affect P_{PA} when administered to pigs with GBS-induced elevation in P_{PA} . Inasmuch as prevention and reversal of GBS-induced pulmonary hypertension are accomplished with the TxA₂ antagonist but not with PAF antagonists, these data suggest that TxA₂, rather than PAF, is responsible for the early pulmonary hypertension in this model of neonatal GBS sepsis. Therefore, TxA₂ antagonists may be clinically useful in treating pulmonary hypertension related to GBS sepsis. (*Pediatr Res* 26: 420-424, 1989)

Abbreviations

GBS, group B Streptococcus
5HETE, 5-hydroxyeicosatetraenoic acid
PAF, platelet-activating factor
 P_{PA} , pulmonary artery pressure
TxA₂, thromboxane A₂

and hypoxemia) include cyclooxygenase products [TxA₂ (5-9)], and leukotrienes (11). Lipoxygenase products [5-HETE, 12-HETE, leukotrienes C₄ and D₄ (10)] may also play a role in the pathogenesis of the late phase pulmonary vascular injury and increased permeability. TxA₂ and leukocytes appear to be important mediators of the pulmonary dysfunction induced by GBS toxin in lambs (33).

PAF is an autocooid phospholipid mediator with a basic structure of 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine. Its cellular sources include endothelial cells, platelets, neutrophils, mast cells, and macrophages; its release can be elicited by multiple inflammatory stimuli (12, 13). Among its many biologic effects is the ability to aggregate platelets and neutrophils (12, 14), and to cause activation of cyclooxygenase and lipoxygenase pathways (12, 23, 35). In whole animals, PAF is capable of inducing pulmonary hypertension, shock, hypoxemia, neutropenia (12, 13), and increased pulmonary vascular and epithelial permeability (14-16, 38). In newborn piglets, PAF-induced pulmonary hypertension is in large part mediated through TxA₂ (35). Because these derangements resemble those produced by endotoxemia, the intravascular release and actions of PAF have been studied and found to be significant in a guinea pig model of Salmonella endotoxemia (17), and in *Escherichia coli* endotoxemia models in sheep (18, 34) and rats (19, 20).

GBS sepsis shares much of the pathophysiology of endotoxemia. As some of its effects are mediated by cyclooxygenase and lipoxygenase products (14, 21-23), in animal models pharmacologic blockade of either eicosanoid significantly attenuates the pathophysiologic abnormalities (5-9, 24) and decreases mortality (25) in this condition.

Thus, we hypothesized that PAF is released during group B streptococcal sepsis, and may be directly or indirectly responsible for some of the pathophysiologic findings, particularly pulmonary hypertension.

MATERIALS AND METHODS

Animal Preparation. Twenty-two piglets (1-14 d old; 1.4-4.5 kg) obtained from a commercial breeder, were anesthetized with ~65% nitrous oxide/35% oxygen/1.5-4% halothane, and intravenous access established via an ear or antecubital vein. Intravenous atropine (0.1 mg) and pancuronium (0.1 mg/kg) were followed by endotracheal intubation. The gas mixture was delivered with a Harvard (South Natick, MA) ventilator (10 mL/kg, 30 breaths/min). The halothane content was reduced to 0.5-1.0% and end-expiratory pressure was 1-3 cm H₂O. A catheter was advanced into the aorta from a common carotid artery. Additional catheters were advanced from the ipsilateral jugular veins into the right heart and pulmonary artery. Aortic, pulmonary arterial, and proximal airway pressures were monitored via Gould P231D pressure transducers (Gould Inc., Oxnard, CA),

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and recorded continuously (Gilson 5/6H multichannel recorder, Gilson Medical Electronics, Middleton, WI). Temperature was monitored with a rectal probe, and controlled with a heating pad and plastic wrap. During the experimental protocol, lasting 4 h, further doses of pancuronium were given if it became necessary to abolish interference of spontaneous respirations with hemodynamic monitoring. An intravenous infusion of Ringer's lactate, with 5% dextrose, was maintained throughout the experiment.

Experimental Protocols. Effect of PAF and U46619 on pulmonary and systemic arterial pressures. In six piglets, pulmonary and systemic pressor response to intravenous PAF (gift of Dr. Bernard Arnoux, INSERM, Paris, France), and the TxA_2 (endo-peroxide) analogue, U46619 (Upjohn, Kalamazoo, MI) was assessed. In addition, we determined the ability of one dose of putative antagonists of PAF (SRI 63072 and SRI 63441) or TxA_2 (SQ 29548) to alter the effect of the above exogenous agonists. The PAF antagonists SRI 63072 (26) and SRI 63441 (27) were gifts of Dr. Dean Handley, Sandoz Research Institute, E. Hanover, NJ. SQ 29548 (28) was a gift of Dr. Martin Ogletree, Squibb Institute, Princeton, NJ. Before use, stock solutions of PAF (1.0 mM in 80% ethanol) were diluted with normal saline in a polypropylene tube to 1.8 μM . Frozen stock saline solutions of U46619 (10 μM) were thawed before use. SRI 63072 was dissolved in distilled water (3 mg/mL) immediately before use; SRI 63441 (1 mg/mL in distilled water) was kept frozen, and aliquots thawed prior to use; a 1 mL/kg dose of each of these solutions was used in the experiments. Each dose of SQ 29548 (1.5 mg/mL in 15% ethanol and 2% calcium carbonate) was 0.75 mg/kg. All stock solutions were kept at -20°C ; each dose was injected as a bolus over 15 s.

In this protocol, a dose of PAF (0.5 nmol/kg) followed 20 min later by U46619 (1 nmol/kg), were administered one to three times to each piglet; the 20-min intervals between injections insured return to baseline of P_{PA} . Either PAF antagonist (SRI 63072, $n = 4$ and SRI 63441, $n = 2$) or the thromboxane antagonist ($n = 6$) were then given, and the response to PAF and U46619 again measured (only the results from first doses after the antagonists are reported). Peak mean P_{PA} was used as the measured parameter, because the dose of PAF used produces only a small, transient decrease in cardiac output in the piglet (Pinheiro JMB, unpublished observations).

Effect of GBS. GBS type Ia-c (strain obtained from the Yale-New Haven Hospital Microbiology Laboratory, clinical isolate from a neonate) were incubated for 24 h on sheep blood agar, at 37°C . The bacterial colonies were suspended in sterile lactated Ringer's with 5% glucose, centrifuged at $4 \times g$, 3°C for 30 min, and the bacterial pellet resuspended in Ringer's; bacterial concentration was adjusted to $2 \times 10^9/\text{mL}$, using a Beckman DU-2 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) and a standard curve of bacterial colony-forming units versus optical density.

The following five experimental protocols were followed to determine the effect of GBS (Fig. 3).

Control ($n = 3$). Animals were pretreated with the thromboxane antagonist vehicle (0.75 mL/kg of 15% ethanol in calcium carbonate) and then 3 mL/kg of lactated Ringer's with 5% dextrose that was processed through sterile sheep blood agar plates was infused intravenously over 1 h.

GBS ($n = 5$). Animals received GBS suspension (see above) for one hour (1×10^8 bacteria/kg/min, or a total of 6×10^9 bacteria/kg). Delivery of the suspension was stopped and the animals followed for an additional 3 h.

PAF antagonists ($n = 6$). Animals were pretreated with either SRI 63072 (3 mg/kg, $n = 3$) or SRI 63441 (1 mg/kg, $n = 3$) and then GBS was administered as discussed previously. The effect of either antagonist was similar and data are combined for clarity.

TxA_2 antagonist ($n = 4$). Animals were pretreated with SQ 29548 (0.75 mg/kg) immediately prior to the GBS infusion (see above).

Table 1. Baseline pulmonary artery pressures before PAF or U46619 boluses*

	PAF experiments	U46619 experiments
Untreated baseline	20.3 \pm 2.4	21.5 \pm 7.7
Post-SRI 63072 baseline	22.5 \pm 5.2	21.0 \pm 8.2
Post-SQ29548 baseline	20.7 \pm 5.7	23.0 \pm 9.8
Post-SRI 63441 baseline	16.0 \pm 0.0	21.0 \pm 0.5

* Mean \pm SEM, in torr; no statistical differences between groups.

Antagonists administered after GBS ($n = 4$). Animals were given SRI 63072 (3 mg/kg) before administration of GBS and again at 10–12 min after initiation of the GBS infusion. Ten min later SQ 29548 (0.75 mg/kg) was given, *i.e.* 20 min into the GBS infusion.

Measurements of airways and vascular pressures and arterial blood gases were made at baseline and 15, 30, 60, 120, 180, and 240 min during GBS (or control) infusion. Additional measurements were made hourly for 3 h after cessation of GBS infusion. Cardiac output determinations were made at baseline, 15 min during GBS and then 1 h after cessation of GBS. Cardiac output was determined from indicator dilution outflow curves using ^3H -benzoyl-phenylalanyl-alanyl-proline, a synthetic substrate for angiotensin-converting enzyme and discontinuous fraction collecting (29).

Data analysis. Data were analyzed using analysis of variance for repeated measures; when significant statistical differences ($p < 0.05$) were present, means between groups were compared using unpaired Student's *t* test. Results are presented as mean \pm SEM.

RESULTS

Effect of PAF and U46619 on pulmonary artery pressure in anesthetized piglet. Baseline P_{PA} were normal and comparable in all groups (Table 1). Injection of PAF produced a rapid and large increase in P_{PA} (see Fig. 1E) and a transient slight decrease in systemic arterial blood pressure. At the peak of the P_{PA} pressor response (approximately 1 min), systemic arterial blood pressure had returned to or exceeded control and in two pigs studied, there was only a slight decrease in cardiac output (data not shown). A total of 15–20 min after the peak response, P_{PA} had returned to baseline. Injection of U46619 (Fig. 1A) produced a similar effect, with the exception that there was only a mild, transient systemic hypertension, without initial hypotension.

Mean values of percent change in maximal P_{PA} response to PAF or U46619 are shown in Figures 2 and 3, respectively. Each agent increased P_{PA} by approximately 160%. SQ 29548 partially reduced the effect of PAF (Figs. 1F and 2) and completely abolished the effect of U46619 (Figs. 1B and 3) on P_{PA} . At the dosages administered, SRI 63072 attenuated the P_{PA} response to PAF (Figs. 1G and 2) but did not significantly affect the response to U46619 (Figs. 1C and 3). SRI 63441 completely abolished the increase in P_{PA} due to PAF (Figs. 1H and 2) and also slightly modified the P_{PA} response to U46619 (Figs. 1D and 3).

Effect of GBS infusion on pulmonary and systemic hemodynamics. There were no significant changes in either PaO_2 , PaCO_2 , or arterial pH with time in any of the five experimental groups. Baseline values were: $\text{PaO}_2 = 211 \pm 8$ torr; $\text{PaCO}_2 = 32 \pm 2$ torr; and arterial pH = 7.50 ± 0.03 .

There was no significant effect of GBS on cardiac output measured during or after infusion in any of the experimental groups. There was a slight, but significant decrease in cardiac output, 15 min and 2 h after control infusions. Cardiac output averaged 419 ± 29 mL/min/kg before infusion of control or GBS suspensions.

Mean systemic arterial pressure was 70 ± 3 torr before control or GBS infusions. There was no statistically significant effects of

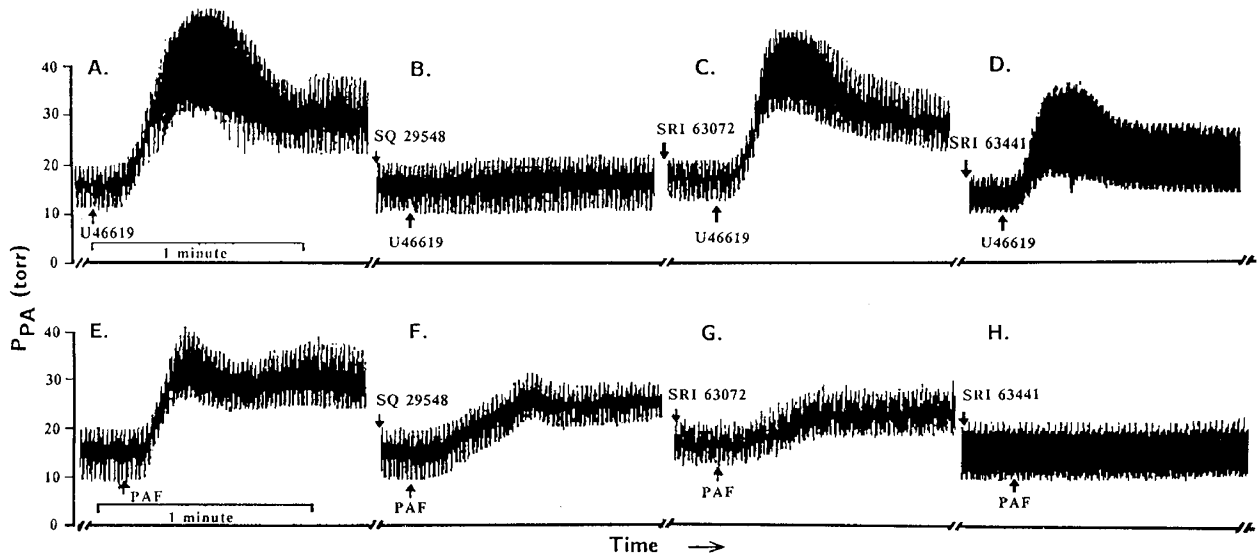


Fig. 1. P_{PA} tracings showing response to boluses of the TxA₂-mimetic U46619, 1 nmol/kg intravenous, and its modification by the TxA₂ antagonist SQ 29548, and PAF antagonists SRI 63072 and SRI 63441 (A to D). E to H demonstrate the corresponding response to PAF, 0.5 nmol/kg intravenous, and its alteration by the same antagonists.

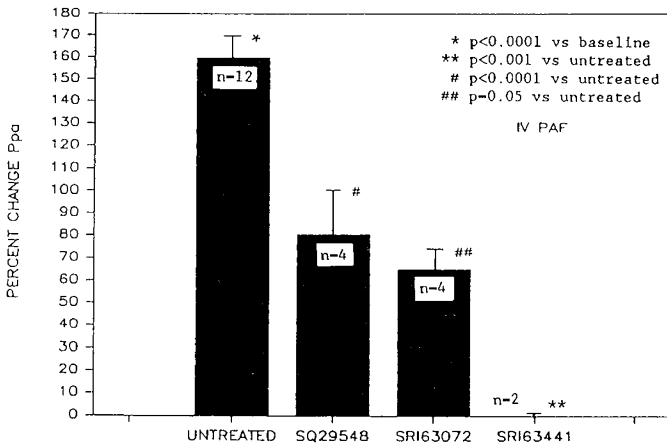


Fig. 2. Mean peak P_{PA} response to PAF boluses, 0.5 nmol/kg intravenous, in piglets untreated or pretreated with TxA₂ antagonist SQ 29548 or PAF antagonists. The significant ($p < 0.01$ versus baseline) increase in P_{PA} with PAF alone was attenuated by SRI 63072 ($p < 0.01$ versus untreated) and SQ29548 ($p = 0.05$), and abolished by SRI 63441 ($p < 0.01$).

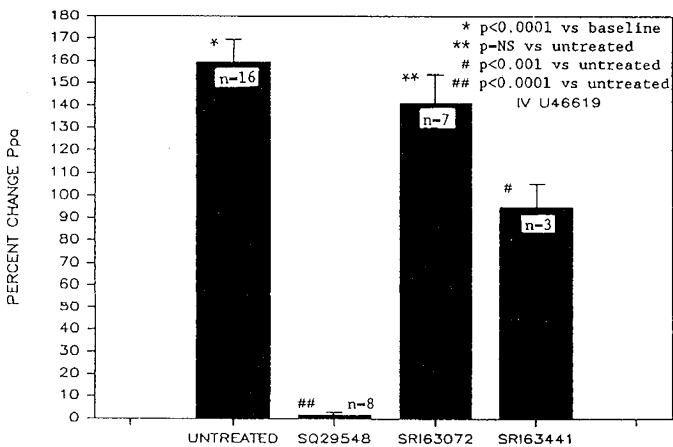


Fig. 3. Mean peak P_{PA} response to U46619 boluses, 1 nmol/kg intravenous, in piglets untreated, or pretreated with TxA₂ antagonist SQ 29548 or PAF antagonists. The increase in P_{PA} ($p < 0.01$ versus baseline) by U46619 alone was attenuated by SRI 63441 ($p = 0.01$ versus untreated), and abolished by SQ29548 ($p < 0.01$).

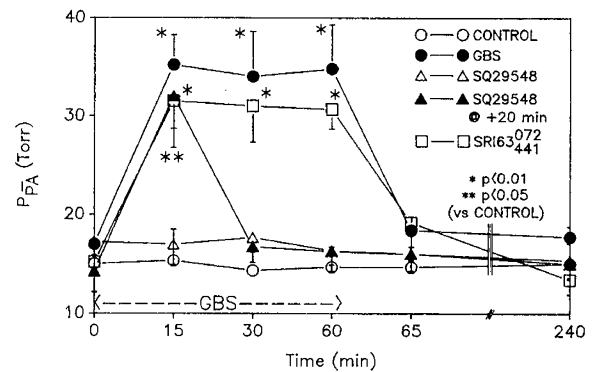


Fig. 4. Mean pulmonary artery pressure changes in each group during the study protocol. P_{PA} increased throughout the GBS infusion; PAF antagonists did not prevent (SRI63072/441) or reverse (group SQ29548 at +20 min) such increase. SQ29548 abolished (SQ29548) and reversed (SQ29548 at +20 min) the pulmonary hypertension. * $p < 0.01$ versus control; ** $p < 0.05$.

either control or GBS infusions on mean systemic arterial pressure in untreated or treated groups.

In Figure 4, mean values of P_{PA} are shown for all five groups. Infusion of GBS led to a prompt and sustained increase in P_{PA}. P_{PA} returned to control values within 5 min of discontinuation of the GBS infusion. Pretreatment of the animals with the SQ 29548 completely inhibited the P_{PA} effect of GBS. In contrast, pretreatment with either SRI 63072 or SRI 63441 did not significantly affect the rise in P_{PA} during GBS infusion. Administration of SRI 63072 during the pulmonary hypertension associated with GBS was without any effect on P_{PA}; however, injection of SQ 29548 promptly reversed the GBS-induced pulmonary hypertension to pre-GBS values of P_{PA}.

DISCUSSION

Our objective in this study was to examine a possible role for PAF in the pulmonary hypertension induced by GBS in the piglet model. Given that PAF administration causes TxA₂ release from platelets and into the circulation of newborn piglets (35) and sheep (38), and that TxA₂ contributes to the bronchial smooth muscle effects of PAF (34), and probably to the newborn lamb's pulmonary vascular response to GBS (33), it was important to evaluate the contribution of TxA₂ to a possible effect of

PAF. A pharmacologic approach, using specific receptor antagonists, allowed us to assess the contributions of each of these circulating mediators to the pulmonary hypertension.

The use of P_{PA} as a marker for the early effects of these mediators in this piglet model is justified as P_{PA} probably reflects pulmonary vascular resistance (cardiac output and systemic arterial pressure are not decreased in either the pharmacologic or GBS infusion experiments); further, we hope to have eliminated the component of hypoxic pulmonary vasoconstriction by maintaining the animals in a mild hyperoxic state throughout the experiments.

Prior work using models of GBS infusion had found contributions of TxA_2 and leukotrienes to the early pulmonary hypertension in GBS sepsis (5, 7, 11). There is now evidence that in the newborn piglet PAF administration causes pulmonary hypertension, which is to a large extent TxA_2 mediated (35). Untested was the possibility that PAF release by GBS might activate the cyclooxygenase and lipoxygenase pathways.

Using P_{PA} and its modification by the receptor antagonists as an endogenous bioassay for the presence of circulating PAF or TxA_2 , we found no evidence to support a role for PAF in producing the pulmonary hypertension in this model of GBS sepsis. Specifically, the PAF antagonists neither prevented nor reversed the pulmonary hypertension after GBS (Fig. 4); furthermore, the P_{PA} elevation was totally abolished by a TxA_2 antagonist, which, in our preliminary experiments, only attenuated the pulmonary hypertension caused by exogenous PAF. The latter finding favors TxA_2 as being solely responsible for the early pulmonary hypertension in this model. This contention is strengthened by the fact that established pulmonary hypertension was rapidly reversed *in toto* by the administration of a TxA_2 antagonist. Accordingly, our study supports the original observations of Sandberg *et al.* (33), using different methods.

Published work on the role of PAF in endotoxemia, although scant, supports the involvement of this mediator in the early phase of the pulmonary vascular response, whether in causing systemic hypotension (19, 20), or pulmonary hypertension and hypoxemia (18, 34). Our data suggest that PAF is not a pivotal mediator of the pulmonary hypertension associated with GBS in neonatal pigs.

Obvious differences in our model, compared to those cited above, include the use of GBS, neonatal pigs, and nonhypoxic conditions; these differences might underlie the insignificant role of PAF in our experiments. However, the pathophysiology of GBS and gram-negative septicemia is quite comparable (3–10). PAF is a ubiquitous molecule, and it has been found in the lungs of human fetuses (30) and neonates (31); one would expect it to have similar functions in piglets as in other mammals. Finally it is unclear whether hypoxemia or tissue hypoxia are necessary for PAF release in the models studied.

If endotoxemia results in enough cell membrane disturbance to activate phospholipase A_2 , one would expect release of ether-linked glycerylphosphocholines [PAF precursors (22)] from the membrane. Some of the PAF thus formed can reach the circulation, where it is a very potent activator of leukocytes, platelets, and endothelial cells (32); however, in sheep, the pulmonary hypertensive, capillary leak, and decreased lung dynamic compliance responses to PAF are independent of platelets and neutrophils (38). PAF is also rapidly inactivated by plasma acetylhydrolase, into biologically inactive lyso-PAF. It is conceivable that rapid inactivation of small amounts of PAF released into the circulation will annul its effects. Thus, one can speculate that significant tissue injury may be necessary to produce pathophysiologically relevant quantities of circulating PAF, which would then amplify multiple mechanisms leading to lung dysfunction and injury. Our data show that the action of circulating PAF is not necessary to produce the early phase pulmonary vascular response.

An important consideration is a possible endothelial-dependent pulmonary vasodilatory effect of PAF. This has been demonstrated only in precontracted rat pulmonary arteries, at low

doses of PAF (36, 37); at higher doses or with normal baseline pulmonary vascular tone, only vasoconstriction is demonstrable (37). Doses as low as 0.01 nmol in normoxic piglets produce only increases in pulmonary vascular resistance (35). In our piglets with normal baseline pulmonary arterial pressures, pulmonary hypertension in response to PAF was not unexpected. However, it is conceivable that circulating PAF might attenuate the vasoconstrictor effects of TxA_2 . Our scant evidence suggests that PAF is not a vasodilator in the baseline state, as administration of the antagonists did not result in pulmonary hypertension; also, PAF antagonism did not potentiate the U46619 or GBS-induced pulmonary hypertension, suggesting that PAF did not have a significant vasodilatory function in this system.

The use of receptor antagonists of circulating mediators, previously unreported in this model, may have significant advantages over less specific cyclooxygenase and lipoxygenase inhibitors. The improved specificity of this approach should help in evaluating the contribution of each mediator to a process. It may also decrease the potential for blocking synthesis of "protective" compounds (*e.g.* Pgl_2), and for shunting precursors toward the formation of potentially harmful lipoxygenase metabolites. Nevertheless, the receptor specificity of PAF and TxA_2/PgH_2 receptor antagonists is technically impossible to demonstrate in systems more complex than a receptor binding assay, due to postreceptor interactions between the two mediator systems. Our pharmacologic data show preferential inhibition of exogenous agonist effects by their respective putative antagonists, as well as some decrease in response to the other agonist. This is compatible with nonspecific binding of the drug, but also explainable by postreceptor phenomena. Indeed, the finding of Bradley *et al.* (35) that SQ 29548 blocks the pulmonary hypertensive response to PAF in piglets, although the resulting rise in TxB_2 was similar, implies that the primary response to PAF (TxA_2 release) was not modified by SQ 29548.

In the absence of PAF measurements, conclusions regarding its unimportant role in this model can only be reached by assuming that total PAF blockade was achieved at the doses of blockers used. Support for that assumption is derived from demonstration of effectiveness at comparable doses in original studies using these drugs (26, 27), and from our own evidence, where PAF effect was abolished at the doses used. We avoided higher doses because of concerns with toxicity; the bipolar nature of the drugs confers them detergent properties, and we had found platelet lysis in *in vitro* assays.

We find no evidence for a role of PAF in this model of GBS endotoxemia, and conclude that TxA_2 is solely responsible for the pulmonary hypertensive response.

Delineation of the mediator mechanisms responsible for the often severe hemodynamic abnormalities in neonatal GBS sepsis has more than academic interest. The recent development of receptor antagonists for PAF and TxA_2 brings to the horizon the age when clinical use of such drugs can modify the body's response to invasion by endotoxins. Further investigation is now necessary to better define the utility of circulating mediator blockade, as well as its possible untoward consequences.

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