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Influence of Thromboxane A_2 Receptor Antagonism on Pulmonary Vasoconstrictor Responses

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ABSTRACT. Thromboxane A_2 (TxA_2) is an arachidonic acid metabolite which causes severe pulmonary vasoconstriction (PV) and may mediate the PV produced by platelet-activating factor (PAF-acether) and leukotriene D_4 (LTD_4). To determine the role of TxA_2 receptors on PAF-acether, LTD_4, and hypoxia-induced PV, we administered PAF-acether 0.1 nmol/kg, the TxA_2 analog U-46619 0.2 μg/kg/min, LTD_4 3.0 μg/kg, or acute hypoxia (FiO_2 = 0.12 for 3 min) before and during the infusion of the selective TxA_2 receptor blocker SQ 29 548 50 μg/kg/min or vehicle into 27 open-chest, anesthetized newborn piglets, measuring pulmonary and systemic arterial pressures, cardiac index, and right and left ventricular pressures and dimensions. Mean pulmonary arterial pressure rose and cardiac index fell in response to PAF-acether (14 ± 1 to 32 ± 2 mm Hg and 91 ± 5 to 15 ± 5 mL/kg/min, both $p < 0.01$), U-46619 (11 ± 1 to 28 ± 2 mm Hg and 93 ± 10 to 36 ± 9 mL/kg/min, both $p < 0.01$), and LTD_4 (13 ± 3 to 22 ± 2 mm Hg and 85 ± 12 to 29 ± 9 mL/kg/min, both $p < 0.05$). Acute hypoxia increased PAP (12 ± 1 to 26 ± 2 mm Hg, $p < 0.01$) but did not alter cardiac index. Infusion of SQ 29 548 prevented PAF-acether and U-46619-induced increases in pulmonary arterial pressure (13 ± 1 to 14 ± 1 mm Hg and 12 ± 1 to 12 ± 1 mm Hg) and decreases in cardiac index (70 ± 4 to 70 ± 3 mL/kg/min and 94 ± 14 to 92 ± 12 mL/kg/min) but failed to alter the response to LTD_4 or hypoxia. Vehicle had no effect. We conclude that TxA_2 receptors are not involved in LTD_4 or hypoxia-induced PV but play an important role in the PV produced by PAF-acether and U-46619. (*Pediatr Res* 26:175–179, 1989)

Abbreviations

LT, cysteinyl leukotrienes
PAF-acether, platelet activating factor (1-0-hexadecyl-2-acetyl-sn-glyceryl phosphocholine)
PV, pulmonary vasoconstriction
Tx, thromboxane
6-k-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$
SVRI, systematic vascular resistance index
MAP, mean systemic arterial pressure
RVEDP, right ventricular end-diastolic pressure
PVRI, pulmonary vascular resistance index
PAP, pulmonary arterial pressure
LVEDP, left ventricular end diastolic pressure

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Vascular tone in the lung is determined by the local balance of vasodilator and vasoconstrictor influences (1). Arachidonic acid (2, 3) and prostacyclin (4) are involved in the regulation of basal tone in the normal pulmonary circulation; TxA_2 (5), PAF-acether (6, 7), and LT (8, 9) have been implicated as mediators of pathophysiologic PV. Released by inflammatory cells, platelets, and vascular endothelium, these membrane lipids appear involved in hypoxic (9–13) and septic (14–17) PV in newborns.

TxA_2 (and its biologically stable prostaglandin endoperoxide analog, U-46619) causes marked PV (5) and acts as potent stimuli for platelet aggregation (18). TxA_2 is necessary for pulmonary venous constriction during hypoxia in isolated lamb lungs, but appears not to mediate hypoxic PV alone (19). TxA_2 release accompanies septic (14–17) and PAF-acether-induced (20) PV in newborn experimental animals and mediates LT response in sheep but not in cats or pigs (8, 21–23). Elevated TxA_2 levels are also present in human infants with persistent pulmonary hypertension (24). Cyclooxygenase and thromboxane synthesis blockade have been shown to attenuate, but not abolish, the pulmonary vasoconstrictor response to group Bβ-hemolytic Streptococcus (14, 16), PAF-acether (20), and LT (19). Neither cyclooxygenase nor thromboxane synthetase blockade has been demonstrated to affect U-46619 induced PV. The development of the highly specific TxA_2 receptor antagonist, SQ 29 548 (19, 25–28) should provide a new means to investigate the role of the TxA_2 receptor on pulmonary vasoconstrictor responses. The present study was undertaken to evaluate the selectivity of the blocking effects of SQ 29 548 in the circulation of the newborn pig and to determine the relative contribution of TxA_2 to PAF-acether, U-46619, LT, and hypoxia-induced PV.

MATERIALS AND METHODS

Preparation. The hemodynamic effects of PAF-acether, U-46619, leukotriene D_4, and hypoxia were assessed in 27 open-chest newborn piglets weighing 2.0–3.9 kg and aged 7–14 d. There were 16 males and 11 females.

Animals were sedated with intraperitoneal sodium pentobarbital (10 mg/kg) and anesthetized with intravenous sodium pentobarbital (30 mg/kg). Each animal was intubated with a 3.5-mm outer diameter endotracheal tube and mechanically ventilated with a Harvard animal respirator (Harvard Apparatus Co., Millis, MA) delivering 3 cm H_2O positive end-expiratory pressure. Inspired oxygen concentration and ventilatory rate were modified at the onset of each experiment to achieve arterial P_{CO_2}

between 35 and 45 mm Hg and P_{O_2} between 100 and 200 mm Hg, to prevent any hypoxic component of agonist administration from independently affecting PAP. Polyvinyl catheters were placed in the left external jugular vein and carotid artery and advanced to the superior vena cava and ascending aorta, respectively. A 4 Fr high-fidelity pressure catheter (Millar Instruments, Houston, TX) was directed from the carotid artery to the left ventricle.

A left lateral thoracotomy was performed in the fourth intercostal space. The pericardium was incised along the main pulmonary trunk and the heart suspended in the pericardial cradle. Polyethylene cannulae were inserted into the right ventricle and main pulmonary artery. Since the ductus arteriosus is not patent in this preparation (20), a calibrated electromagnetic flow transducer (North Carolina Instruments, King, NC) was placed around the main pulmonary artery to measure cardiac output. Two pairs of 1-mm ultrasonic crystals (Dimension 3, Cardiff-by-Sea, CA) were positioned 10 mm apart and embedded 1–2 mm beneath the anterior epicardial surface of the right and left ventricles. Myocardial segment length was recorded continuously from each region using appropriate signal processing instrumentation (Sonomicrometer 120, Triton Technology, San Diego, CA) and previously described techniques (20). Vascular pressures were measured with pressure transducers (model P23 ID; Gould-Statham Instruments, Hato Rey, PR) and recorded on a multichannel recorder (model 7758A, Hewlett-Packard Instruments, Rockville, MD). Surface electrocardiogram was recorded for heart rate.

Baseline hemodynamic measurements were taken and determined to be stable for at least 1 h after the surgery in all animals. During the stabilization period, all animals received 0.9% NaCl 10 mL/kg.

Protocol 1 (n = 13). After base-line arterial blood gas determination and hemodynamic measurements, blood was sampled for levels of TxB_2 and 6-k-$PGF_{1\alpha}$, the stable metabolites of TxA_2 and prostacyclin, respectively. Animals then received PAF-acether 0.1 nmol/kg intravenous bolus using a standardized 2-s injection technique. This dose of PAF-acether was chosen to produce reversible, near-maximal hemodynamic changes based on previous experiences in our laboratory (20). All parameters were recorded continuously. One min after PAF-acether injection (peak effect), TxB_2 and 6-k-$PGF_{1\alpha}$ levels were repeated. Animals were allowed up to 30 min to recover. Baseline parameters were recorded, and piglets were then ventilated with an inspired gas concentration of 12% O_2, 88% N_2 for 3 min. At 3 min, arterial blood was sampled for pH, P_{CO_2} and P_{O_2} measurement. Hypoxia was then discontinued. Next, animals were randomized to receive either SQ 29 548 (SQ), 50 μg/kg/min by continuous intravenous infusion (n = 7), or an equal volume of the ethanol containing vehicle (n = 6). The dose of SQ was based on previous *in vivo* studies that showed maximal antagonistic capacity in dogs, cats, and pigs (25–28). Then 30 min later, hypoxia and PAF-acether were given in random order. At the conclusion of the study, animals were given a sodium pentothal overdose and a lethal KCl injection.

Protocol 2 (n = 10). To confirm the specificity of SQ for the TxA_2 receptor in our preparation, a group of six piglets received, in random order, the stable TxA_2 analog U-46619, 0.2 μg/kg/min intravenous infusion, and LTD_4, 3.0 μg/kg intravenous bolus. The dose of U-46619 was chosen (based on preliminary data) to approximate the hemodynamic response to PAF-acether (*i.e.* ≥50% rise in mean pulmonary arterial pressure); the amount of LTD_4 was based on previous dose-response studies in our laboratory (29). Each drug administration was separated by a recovery period of at least 15 min. When circulatory status returned to baseline (2–15 min), animals were given the hypoxic challenge as described in group 1. After a 30-min recovery period, SQ infusion was begun. Then 30 min later, administration of U-46619, LTD_4, and hypoxia were repeated.

To determine the efficacy of TxA_2 receptor antagonism on the initial exposure to PAF-acether, U-46619, and hypoxia, a separate group of piglets (n = 4) was given SQ without prior agonist challenge. Baseline hemodynamic measurements were obtained before and 30 min after SQ initiation. Animals then received PAF-acether, U-46619, and hypoxia, as detailed above, in random order.

Protocol 3 (n = 4). To determine the effects of sustained exposure to PAF-acether and to evaluate reversibility of more extended PAF-acether action by TxA_2 receptor blockade, four piglets received PAF-acether 0.1 nmol/kg/min by continuous intravenous infusion for 15 min. Animals were allowed a 60-min recovery period. Two animals were assigned to receive SQ and two were given only vehicle, as described above, followed by repeat agonist infusion. One vehicle-treated animal died in the first 60 s of repeat PAF infusion; one experimental animal died shortly after SQ initiation, prior to receiving PAF-acether.

Drugs. Pure synthetic PAF-acether (1-0-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine, kindly provided by Dr. F. Snyder, Oak Ridge Associated Universities, Oak Ridge, TN) was dissolved in 0.9% NaCl to a concentration of 1 nmol/10 μL and aliquots kept frozen (−70°C) until used. Each aliquot was thawed only once and diluted with 0.9% NaCl to a final volume of 100 μL/injection. A stable TxA_2 analog, the prostaglandin endoperoxide U-46619 (kindly provided by Upjohn Diagnostics, Kalamazoo, MI), was dissolved in absolute ethanol and was stored frozen. Solutions of U-46619 for injection were freshly prepared in 0.9% NaCl. Pure synthetic LTD_4 (kindly provided by Dr. J. Rokach, Merck Frosst, Dorval, Canada) was stored as a stock solution at −70°C. On the day of an experiment an aliquot of the stock solution was thawed once and diluted with 0.9% NaCl to a final volume of 100 μL for injection into the animal. A stock solution of SQ 29 548 (1S-[1α,2β(5Z),3β,4α])-7-[3-(2-[(phenylamino) carbonyl] hydrazino methyl-7-oxabicyclo-[2,2,1] hept-2-yl]-5-heptanoic acid, generously supplied by Dr. M. Ogletree, Squibb, Princeton, NJ) was prepared by dissolving 15 mg in 2.5 mL of absolute ethanol and stored in the freezer. Solutions for infusion were prepared daily by a 1:10 dilution of the stock by 2 mM Na_2CO_3 in 0.9% NaCl.

TxB_2 and 6-k-$PGF_{1\alpha}$ RIA. Blood samples (1.5 mL) were drawn from the external jugular vein, collected into heparinized (100 U/mL) syringes, and immediately decanted into tubes containing a final concentration of indomethacin 20 μM. They were centrifuged for 1 min. The plasma was harvested and frozen at −70°C for later analysis by RIA, as detailed previously (30).

Data analysis. All data were continuously recorded; those from mid- to end-expiration were chosen for analysis; the respirator was not stopped at any point. Pulmonary flow was assumed to approximate cardiac output (mL/min). CI was calculated as cardiac output/wt (kg); SVRI was calculated as 80 × (MAP − RVEDP)/CI; and the PVRI was calculated as 80 × (PAP − LVEDP)/CI. Ventricular segment lengths and shortening fractions were assessed using standard methods (31).

Data are presented as mean values ± SEM for the indicated number of piglets. One-way analysis of variance followed by the Student-Newman Keuls test for multiple comparison was used for statistical evaluation. Differences were considered significant when $p < 0.05$.

RESULTS

Protocol 1: effects of PAF-acether before and after SQ or vehicle infusion (Fig. 1). Initial bolus injection of PAF-acether (n = 13) produced significant increases in RVEDP (3 ± 0 to 8 ± 1 mm Hg, $p < 0.01$), PAP (14 ± 1 to 32 ± 2 mm Hg, $p < 0.01$), and PVRI (9.1 ± 1.4 to 340.4 ± 66.2 dynes s cm^{-5} · kg × 10^{-3}, $p < 0.01$). PAF-acether decreased CI (91 ± 5 to 15 ± 5 mL/kg/min, $p < 0.01$), MAP (62 ± 4 to 37 ± 4 mm Hg, $p < 0.01$), left and right ventricular shortening fraction (14 ± 2 to 7 ± 1% and 19 ± 1 to 8 ± 2%, respectively, both $p < 0.05$), and the ratio SVRI/

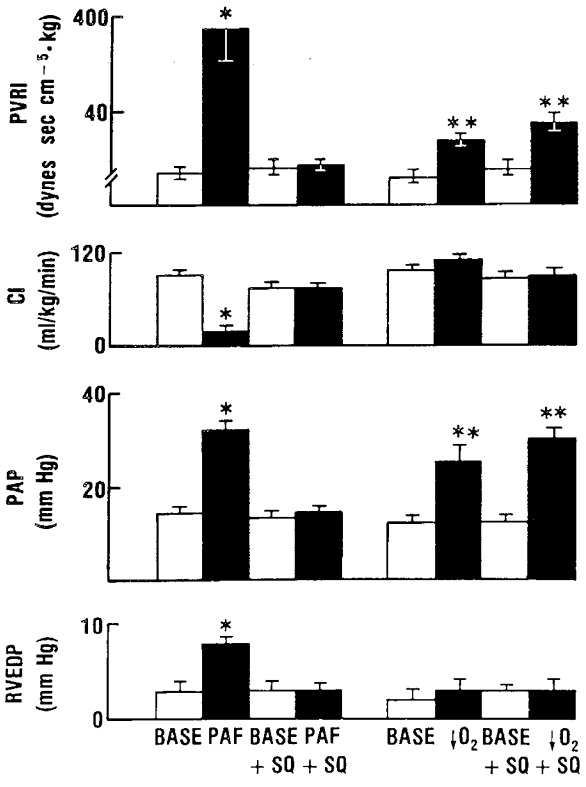

Fig. 1. Influence of TxA_2 receptor antagonism on pulmonary vasoconstrictor responses to PAF-acether and hypoxia. Both PAF-acether 0.1 nmol/kg and hypoxia caused significant rises in PAP and PVRI when compared to baseline; PAF administration also caused RVEDP to increase and CI to decrease. Baseline values were not significantly different before and after infusion of the TxA_2 receptor blocker, SQ 29 548. TxA_2 receptor antagonism completely blocked PAF-acether-induced pulmonary vasoconstriction and the related fall in CI; hypoxic pulmonary vasoconstrictor responses remained intact. Results are expressed as absolute values ± SEM. PVRI data are shown on a logarithmic scale. *BASE*, baseline; *BASE + SQ*, baseline during SQ 29 548 infusion; *CI*, cardiac index; *PAF*, platelet-activating factor; *PAF + SQ*, platelet-activating factor during SQ 29 548 infusion; *PAP*, mean pulmonary arterial pressure; *PVRI*, pulmonary vascular resistance index × 10^{-3}; *RVEDP*, right ventricular end-diastolic pressure; *TxA_2*, thromboxane A_2; *$\downarrow O_2$*, hypoxia; *$\downarrow O_2$ + SQ*, hypoxia during SQ 29 548 infusion. *$p < 0.05$ *versus* BASE; **$p < 0.01$ *versus* BASE + SQ.

PVRI (7 ± 1 to 1 ± 0, $p < 0.01$). LVEDP was unchanged. The pulmonary vasoconstrictor response produced by PAF-acether peaked 15–17 s after injection. Circulatory status returned to baseline within 15 min. In the two animals where TxB_2 and 6-k-$PGF_{1\alpha}$ were measured, TxB_2 rose 440% (10 to 54 pg/100 μL and 6-k-$PGF_{1\alpha}$ increased 650% (8 to 60 pg/100 μL). SQ infusion did not alter baseline MAP (61 ± 5 *versus* 55 ± 4 mm Hg) or PAP (13 ± 1 *versus* 13 ± 1 mm Hg) when compared to vehicle infusion. There was a slight decrease in baseline CI in the experimental group from 80 ± 3 mL/kg/min at the onset of the study to 70 ± 4 mL/kg/min with SQ infusion. Similarly, there was a small decrement in base-line CI in vehicle-treated controls from 102 ± 8 to 97 ± 6 mL/kg/min. Baseline CI was significantly lower in experimental *versus* control animals both initially (80 ± 3 *versus* 102 ± 8 mL/kg/min) and during infusion of SQ (70 ± 4 *versus* 97 ± 6), both $p < 0.05$. TxA_2 receptor antagonism with SQ completely inhibited the PAF-acether-induced rise in RVEDP, PAP, and PVRI and fall in CI (Fig. 1). SQ infusion also prevented the PAF-acether-induced decline in right (17 ± 3 to 17 ± 3%) and left ventricular shortening fraction (10 ± 1 to 10 ± 0%). After SQ TxB_2 and 6-k-$PGF_{1\alpha}$ levels also rose in response to the second dose of PAF-acether (13 to 62 and 13 to 31 pg/100 μL). The ethanol-containing vehicle for SQ did not significantly attenuate PAF-acether-induced hemodynamic derangements (PAP: 13 ± 1 to 34 ± 2 mm Hg; CI: 97 ± 6 to 29 ± 13 mL/kg/min, both $p < 0.01$).

Effects of hypoxia before and during SQ or vehicle infusion (Fig. 1). Initial administration of hypoxia ($n = 13$) produced marked hypoxemia without altering pH or P_{CO_2} (pH 7.41 ± 0.01 P_{CO_2} 38 ± 1 P_{O_2} 179 ± 13 *versus* pH 7.41 ± 0.01 P_{CO_2} 38 ± 1 P_{O_2} 39 ± 2). Hypoxia produced increases in PAP (12 ± 1 to 26 ± 2 mm Hg, $p < 0.01$) and PVRI (7.7 ± 1.0 to 18.6 ± 3.2 dynes s cm^{-5} · kg × 10^{-3}, $p < 0.01$). These values peaked at 3 min and returned to base line within 7 min of discontinuation of hypoxia. The ratio SVRI/PVRI fell (8 ± 2 to 2 ± 0, $p < 0.01$). All other measured parameters were unchanged. Neither SQ nor vehicle infusion altered baseline arterial blood gas content, PAP (11 ± 1 *versus* 10 ± 1 mm Hg), or MAP (64 ± 5 *versus* 58 ± 5 mm Hg). Infusion of SQ failed to block hypoxia-induced pulmonary vasoconstriction (PAP:12 ± 1 to 30 ± 2 mm Hg, PVRI:10.2 ± 2.0 to 27.2 ± 3.7 dynes s cm^{-5} · kg × 10^{-3}, both $p < 0.01$, Fig. 1).

Protocol 2: specificity studies—effects of U-46619 and LTD_4 before and during SQ infusion (Fig. 2; Table 1). Infusion of U-46619 ($n = 6$) caused rises in RVEDP (2 ± 1 to 6 ± 1 mm Hg,

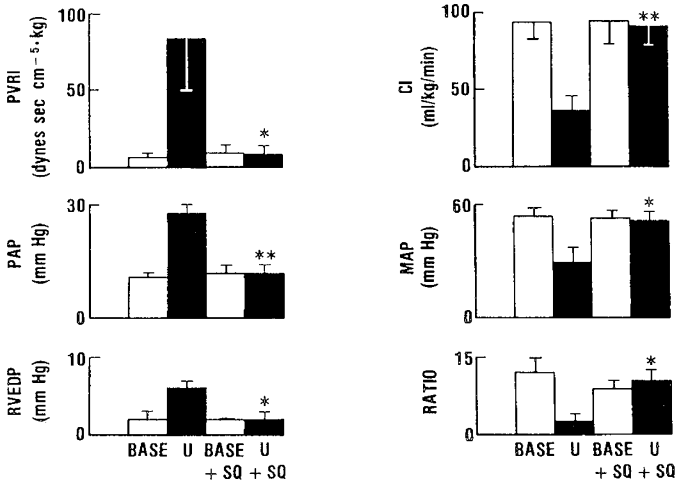

Fig. 2. Circulatory effects of U-46619 before and during SQ 29 548 infusion. U-46619 0.2 μg/kg/min was given to six animals. Initial infusion of U-46619 caused significant rises in PAP, PVRI, and RVEDP and falls in CI, MAP, and RATIO (see text). Administration of the TxA_2 receptor antagonist SQ 29 548 completely inhibited U-46619-induced hemodynamic derangements. Values are ± SEM. Abbreviations as in Figure 1. *MAP*, mean arterial pressure; *RATIO*, ratio of systemic vascular resistance index/pulmonary vascular resistance index; *U*, U-46619; *U + SQ*, U-46619 during infusion of SQ 29 548. *$p < 0.05$ *versus* U, **$p < 0.01$ *versus* U.

Table 1. *Hemodynamic effects of leukotriene D_4 before and during infusion of SQ 29,548 (SQ)**

	Leukotriene D_4		Leukotriene D_4 + SQ	
	Base	Peak	Base	Peak
PAP (mm Hg)	13 ± 3	22 ± 2†	13 ± 1	19 ± 3†
CI (mL/kg/min)	85 ± 12	29 ± 9†	100 ± 8	49 ± 10†
LVEDP (mm Hg)	4 ± 2	13 ± 5†	4 ± 3	13 ± 4†
LVSF (%)	14 ± 2	4 ± 1†	15 ± 2	4 ± 2†

* Values are absolute values ± SEM at baseline and 1 min after leukotriene D_4 injection, before and during infusion of the thromboxane A_2 receptor blocker, SQ 29 548. SQ 29 548 did not significantly alter the circulatory response to leukotriene D_4. LVSF = left ventricular shortening fraction.

† $p < 0.05$ *versus* respective baseline.

$p < 0.05$), PAP (11 ± 1 to 28 ± 2 mm Hg, $p < 0.01$), and PVRI (6.0 ± 1.5 to 83.3 ± 33.8 dynes s cm^{-5} · kg × 10^{-3}, $p < 0.05$), as well as falls in CI (93 ± 10 to 36 ± 9 mL/kg/min, $p < 0.01$), MAP (55 ± 3 to 30 ± 7 mm Hg, $p < 0.05$), and the ratio of SVRI/PVRI (12 ± 3 to 2 ± 1, $p < 0.05$). Right ventricular shortening fraction also declined (15 ± 1 to 11 ± 1%, $p < 0.05$). Left ventricular shortening fraction and LVEDP did not change. The peak PAP induced by U-46619 occurred 1 min after infusion was started. PAP decreased to 22 ± 3 mm Hg by 5 min after infusion was ended, and returned to baseline levels within 10 ± 2 min. SQ infusion totally blocked U-46619-induced PV and the resultant decrement in CI and MAP (Fig. 2).

Initial injection of LTD_4 ($n = 5$) caused significant rises in PAP (13 ± 3 to 22 ± 2 mm Hg, $p < 0.05$), PVRI (9.5 ± 2.9 to 46.3 ± 17.2 dynes s cm^{-5} · kg × 10^{-3}, $p < 0.05$), and LVEDP (4 ± 2 to 13 ± 5 mm Hg, $p < 0.05$). CI and left ventricular shortening fraction fell in response to LTD_4 (from 85 ± 12 to 29 ± 9 mL/kg/min and from 14 ± 2 to 4 ± 1%, both $p < 0.05$). The circulatory effects of LTD_4 peaked at 1 min after injection and dissipated in 7 ± 1 min. TxA_2 receptor antagonism failed to attenuate LTD_4-induced hemodynamic derangements (Table 1).

Effects of pretreatment with TxA_2 receptor blocker on pulmonary vasoconstrictor responses. Infusion of SQ for 30 min did not significantly alter CI (118 ± 2 *versus* 113 ± 4 mL/kg/min), left ventricular shortening fraction (13 ± 1 *versus* 13 ± 1%) or arterial blood gas content when compared to baseline (pH 7.37 ± 0.03, P_{CO_2} 38 ± 5 mm Hg, P_{O_2} 127 ± 13 mm Hg *versus* pH 7.40 ± 0.02, P_{CO_2} 43 ± 3 mm Hg, P_{O_2} 120 ± 6 mm Hg). Pretreatment with SQ eliminated both PAF-acether and U-46619-induced hemodynamic derangements although not affecting hypoxic PV (Table 2).

Protocol 3: effects of sustained PAF-acether infusion with and without TxA_2 receptor antagonism (Fig. 3). Continuous PAF-acether infusion for 15 min increased PAP to a comparable degree as bolus injection (13 ± 2 to 33 ± 2 *versus* 14 ± 2 to 32 ± 2 mm Hg, Fig. 3). Interestingly, unlike bolus injection, sustained PAF-acether-infusion did not significantly decrease CI, MAP (59 ± 10 to 55 ± 9 mm Hg), or right or left ventricular shortening fractions (17 ± 3 to 17 ± 2 and 11 ± 1 to 10 ± 1%, respectively). Treatment with SQ 29 548 prevented PAF-acether induced pulmonary vasoconstriction (PAP: 14 to 13 mm Hg, PVRI: 3.6 to 2.9 dynes s cm^{-5} · kg × 10^{-3}) although vehicle administration had no effect (PAP: 18 to 29 mm Hg, PVRI: 12.9 to 17.8 dynes s cm^{-5} · kg × 10^{-3}, Fig. 3).

DISCUSSION

Results of the present study demonstrate that the pulmonary vascular responses to PAF-acether and the TxA_2 mimic, U-46619, in the open-chest newborn piglet are markedly reduced by the TxA_2 receptor antagonist, SQ 29 548. By contrast, the pulmonary vasoconstrictor response to acute hypoxia and LTD_4 is preserved. The hemodynamic changes induced by selected doses of PAF-acether or U-46619 were severe, much greater than the changes induced in response to selected exposure to LTD_4 or hypoxia. Thus, the capacity of SQ 29 548 to prevent the actions of PAF-acether or U-46619 cannot be attributed to the mildness of response to those agonists. The data suggest that, in our preparation, the TxA_2 receptor is involved in the pulmonary vasoconstrictor actions of PAF-acether and U-46619, but not in those of LTD_4 or hypoxia.

We have previously reported PAF-acether to be an extremely potent pulmonary vasoconstrictor in open-chest, anesthetized newborn piglets. Recent work from our laboratory also suggested a pivotal role for TxA_2 as a mediator of PAF-acether action: cyclooxygenase blockade with indomethacin attenuated the pulmonary vasoconstrictor response to PAF-acether; LTD_4/E_4 receptor antagonism had no effect (20). In contrast, Soifer and Schreiber (32) reported both indomethacin and the LT antagonist, FPL 57231, to decrease PAF-acether-induced pulmonary hypertension in newborn lambs. The dose of PAF-acether chosen for the present study, 0.1 nmol/kg (50 ng/kg) was similar to that described by other investigators in pigs, dogs, and lambs (7, 32, 33) and produced a comparable rise in PAP and PVRI. Unlike its action in the lamb, PAF-acether injection in our piglets caused marked decreases in cardiac output and systemic arterial pressure. Diminished response to PAF-acether during SQ 29 548 cannot be attributed to tachyphylaxis because diminution of response did not occur during control infusions with vehicle for SQ 29 548. In addition, SQ 29 548 blocked PAF-acether-induced pulmonary vasoconstriction in the group without prior exposure to PAF-acether. Taken together, these results support the hypothesis that PAF-acether in piglets is mediated by stimulation of TxA_2 receptors. This interpretation depends on the specificity of the blocking effects of SQ 29 548.

SQ 29 548 has proven a selective and potent TxA_2 receptor antagonist in a variety of test systems. It does not alter cyclooxygenase, thromboxane synthetase, prostacyclin synthetase, or adenylate cyclase activity *in vitro* or *in vivo* (25). SQ 29 548 has been shown to have no effect on systemic concentrations of TxB_2 or 6-k-$PGF_{1\alpha}$ in dogs (26) and similarly had no significant effect

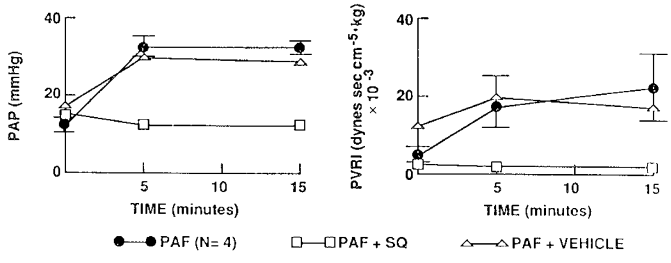

Fig. 3. Pulmonary vascular effects of sustained PAF-acether infusion before and during infusion of SQ 29 548 or vehicle. PAF-acether 0.1 nmol/kg/min was infused into four animals for 15 min and caused PAP and PVRI to rise. TxA_2 receptor antagonism with SQ 29 548 completely blocked PAF-acether-induced pulmonary vasoconstriction whereas vehicle had no effect. Results are expressed as absolute values ± SEM. Abbreviations as in Figure 1.

Table 2. *Effect of SQ 29 548 pretreatment on pulmonary vasoconstrictor responses in piglets (n = 4)*

	PAF-acether		U-46619		Hypoxia	
	Base	Peak	Base	Peak	Base	Peak
PAP (mm Hg)	17 ± 3	20 ± 3	16 ± 2	18 ± 3	12 ± 4	32 ± 1†
PVRI (dynes s cm^{-5}·kg) × 10^{-3}	8.9 ± 1.9	10.5 ± 1.7	8.0 ± 1.3	8.8 ± 2.1	7.4 ± 1.2	18.4 ± 2.3†
CI (mL/kg/min)	122 ± 10	118 ± 19	113 ± 11	119 ± 8	114 ± 5	127 ± 8
RVEDP (mm Hg)	2 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	3 ± 1
RVSF (%)	13 ± 3	14 ± 3	14 ± 3	14 ± 2	14 ± 2	12 ± 4

* Values are absolute values ± SEM at baseline and at peak effect. Pretreatment with SQ 29,548 eliminated PAF-acether and U-46619 circulatory derangements but did not diminish the pulmonary vasoconstrictor response to hypoxia. Abbreviations: RVSF = right ventricular shortening fraction, others as per text.

† $p < 0.01$ *versus* respective baseline.

on the generation of these TxA_2 or prostacyclin metabolites in the current study.

Underwood *et al.* (28) showed that SQ 29 548, 0.5 mg/kg intravenous bolus, eliminated U-46619-induced bronchoconstriction but failed to block the constrictor airway response to prostaglandins $F_{2\alpha}$ and D_2, 5-hydroxytryptamine, metacholine, and histamine (28). Our study in piglets showed that a similar dose of SQ 29 548 markedly diminished the substantial circulatory actions of PAF-acether and U-46619 while having little effect on the more modest vasoconstrictor responses to LTD_4 and hypoxia. We have previously demonstrated that there is no tachyphylaxis to LTD_4 in our preparation (29). Thus, it seems likely that LTD_4 action in the piglet is not mediated by TxA_2 release. These results contrast to findings in mature sheep, where TxA_2 mediates both the pulmonary hemodynamic and lung lymph responses to LTD_4 (8, 21).

The finding that SQ 29 548 not only fails to block, but slightly enhances, the pulmonary pressor response to hypoxia (Fig. 1) is interesting and agrees with results in isolated blood-perfused lamb lungs (19). In the *in vitro* studies, untreated control lungs demonstrated both arterial and venous constriction during hypoxia; addition of indomethacin, Dazmegrel (a thromboxane synthetase inhibitor) or SQ 29 548 to the perfusate abolished hypoxia-induced pulmonary venous constriction and accentuated pulmonary arterial constriction. The observed enhancement of arterial constriction *in vitro* was thought to be due to shunting of arachidonic acid preferentially to the lipoxygenase pathway. A similar mechanism should not account for our results: Hammerman *et al.* (15) in a similar model have shown that TxB_2 levels do not rise in response to hypoxia alone. Moreover, the TxA_2 receptor blocker SQ 29 548 had no inhibitory action on synthesis of cyclooxygenase products.

In conclusion, our results suggest that TxA_2 receptor activation is involved in the pulmonary vasoconstrictor response to both PAF-acether and U-46619, but not to LTD_4 and hypoxia. SQ 29 548 may be a useful pharmacologic tool for studying the role of TxA_2 in pathophysiologic processes in the circulation of the newborn piglet.

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