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Perivascular fluid cuffs decrease lung compliance by increasing tissue resistance

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Abstract

Objective—Lung inflammation causes perivascular fluid cuffs to form around extra-alveolar blood vessels; however, the physiologic consequences of such cuffs remain poorly understood. Herein, we tested the hypothesis that perivascular fluid cuffs, without concomitant alveolar edema, are sufficient to decrease lung compliance.

Design—Prospective, randomized, controlled study.

Setting—Research laboratory.

Subjects—One hundred twenty male CD40 rats.

Interventions—To test this hypothesis, the plant alkaloid thapsigargin was used to activate store-operated calcium entry and increase cytosolic calcium in endothelium. Thapsigargin was infused into a central venous catheter of intact, sedated, and mechanically ventilated rats.

Measurements—Static and dynamic lung mechanics and hemodynamics were measured continuously.

Main Results—Thapsigargin produced perivascular fluid cuffs along extra-alveolar vessels but did not cause alveolar flooding or blood gas abnormalities. Lung compliance dose-dependently decreased after thapsigargin infusion, attributable to an increase in tissue resistance that was attributed to increased tissue damping and tissue elastance. Airway resistance was not changed. Neither central venous pressure nor left ventricular end diastolic pressure was altered by thapsigargin. Heart rate did not change, although thapsigargin decreased pressure over time sufficient to reduce cardiac output by 50%. Infusion of the type 4 phosphodiesterase inhibitor, rolipram, prevented thapsigargin from inducing perivascular cuffs and decreasing lung compliance. Rolipram also normalized pressure over time and corrected the deficit in cardiac output.

Conclusions—Our findings resolve for the first time that perivascular cuff formation negatively impacts mechanical coupling between the bronchovascular bundle and the lung parenchyma, decreasing lung compliance without impacting central venous pressure.

Keywords

permeability; endothelium; thapsigargin; store-operated calcium entry; acute respiratory distress syndrome

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The pulmonary endothelium normally serves as a tightly regulated barrier between blood and interstitium. However, endothelial barrier function is compromised in the blood vessels of critically ill patients with acute lung injury or acute respiratory distress syndrome (ARDS). Endothelial cell barrier disruption causes fluid accumulation in the lung parenchyma, which ultimately leads to inefficient gas exchange and decreased alveolar ventilation, and also in perivascular spaces surrounding extra-alveolar arteries and veins (1). Traditional thinking advocates that capillary endothelial cell barrier disruption increases interstitial fluid accumulation that is pulled by negative interstitial pressure into perivascular spaces of larger vessels as a safety mechanism that limits alveolar flooding. However, recent acute lung injury and pulmonary edema animal models indicate that extra-alveolar vessels exhibit greater permeability responses than do capillaries, both at baseline (2–4) and in response to a variety of inflammatory stimuli (5–9). Furthermore, these experimental findings illustrate that fluid can directly traverse across extra-alveolar vessels and accumulate in perivascular cuffs (7). Whereas some inflammatory agonists exert their actions on extra-alveolar endothelium, others disrupt the capillary endothelial cell barrier and cause alveolar flooding in the absence of perivascular cuffs (6,10). Thus, it appears that perivascular cuff formation and alveolar flooding represent distinct physiologic processes that are regulated by inflammatory agonists that discriminate among different endothelial cell phenotypes along the pulmonary circulation. At the present time, the physiologic significance of perivascular cuff formation and its contribution to lung mechanics are incompletely understood.

Lung compliance is decreased in patients with ARDS. We questioned whether perivascular cuffs reduce either dynamic or static compliance, because several lines of evidence suggest that alveolar fluid filling does not fully explain the decrease in compliance that accompanies ARDS. Surfactant replacement in ARDS patients does not increase tidal volume or decrease peak pressure (11), suggesting that mechanisms other than surfactant inactivation lead to the mechanical changes in lungs of ARDS patients (11). During experimentally induced hydrostatic edema, pulmonary compliance decreases as fluid accumulates in perivascular cuffs, before fluid accumulation in the alveoli (12). Furthermore, studies showing increased airway resistance in animal models of pulmonary edema (13–15) and expiratory flow limitation in ARDS patients (16) suggest that air flow may be decreased in conduit airways in lungs with interstitial fluid accumulation. Thus, alveolar flooding is not sufficient to explain the mechanical changes seen in ARDS-afflicted lungs.

We have previously demonstrated that inflammatory agonists that increase permeability across extra-alveolar vascular segments decrease dynamic compliance, whereas agonists that increase permeability across capillary segments do not change dynamic compliance (10). Because dynamic compliance is determined by airway and tissue resistances, in the current study we sought to determine whether permeability across extra-alveolar vessels, and the resultant perivascular cuffs, decreases either airway or tissue resistances in the intact animal. Our findings indicate that perivascular cuff formation decreases both dynamic and static compliance because of an increase in tissue resistance but not an increase in airway resistance.

MATERIALS AND METHODS

Surgery and Experimental Protocol

All studies were performed using CD40 rats (weight range, 250 – 450 grams) in compliance with the University of South Alabama Institutional Review Board for the care of animal subjects, in accord with the National Institutes of Health guidelines for the ethical treatment of animals. Rats were sedated with intraperitoneal ketamine (5 mg/kg) and xylazine (40 mg/kg) and were intubated via tracheal cut-down and connected to a small animal ventilator

(Scireq, Montreal, Canada). One canula (PE-50) was placed in the jugular vein. A pressure-sensing catheter (Millar, Houston, TX) was also placed in the jugular vein and advanced approximately 4 cm. A volume- and pressure-sensing catheter (Millar) was placed via the carotid artery into the left ventricle. Central venous pressure and left ventricular pressure and volume were amplified and recorded by a Power Lab (Colorado Springs, CO) system connected to a laptop computer. Real-time cardiac volume and heart rate were determined using PVAN software (Millar). A cannula (PE-10) was also placed in the femoral artery to allow arterial blood gas sampling. Rats were ventilated with an 8-mL/kg tidal volume at a respiratory rate of 40 to 50 breaths per minute. After surgery, 15 mins were allowed to pass before the beginning of the experiment. At 15 mins, rolipram (15 μ M, based on blood volume of 7 mL/100 gram body weight) or vehicle control (dimethyl sulfoxide at 10 μ L/100 gram body weight) was injected via the central vein. Ten minutes were allowed to pass before baseline arterial blood gas analysis and assessment of respiratory mechanics were performed. Thapsigargin (0.7 μ M) or vehicle control (dimethyl sulfoxide at 5 μ L per 100 gram body weight) was then administered. Twenty minutes after thapsigargin or dimethyl sulfoxide, respiratory mechanics and blood gases were assessed for a second time. At this point in the experiment, in five rats in each group, heart and lungs were removed *en bloc* and lung tissue was fixed in paraformaldehyde. The experiment was continued in another five rats in each group and albuterol was administered by nebulization (1 mg/mL $\times$ 2 mins). Respiratory mechanics were then measured for a third time in this group.

Evan's Blue Dye Tracer Experiments

To determine whether thapsigargin increases alveolar fluid accumulation, tracer experiments were performed using Evan's blue dye conjugated to albumin as previously described (17,18). Animals were anesthetized as described in Surgery and Experimental Protocol in this article, and jugular vein catheters were placed. Evan's blue dye albumin was slowly (over the course of 5 mins) introduced into the jugular vein (20 mg/kg of body weight, 5% bovine serum albumin) and allowed to recirculate for 10 additional minutes. Thapsigargin was then slowly introduced at the indicated concentrations and allowed to recirculate for a total of 15 mins, after which time two bronchoalveolar lavages (3 mL saline) were performed. Evan's blue dye-albumin in the bronchoalveolar lavage fluid was measured using a ThermoSpectronic Helios Y spectrophotometer at an excitation wavelength of 620 nm.

Bacterial Culture and Inoculation

The PA103pcrV pUCPexoY bacterial strain was used for this study and was provided by Dr. Dara W. Frank (19). Bacteria was grown, maintained, and counted as previously described (20). Animals were anesthetized and placed on a surgical table suspended at an approximate 75° angle. An incision was made immediately above the trachea, and the trachea was exposed by blunt dissection. Bacteria (5×10^7) were introduced into the trachea in 1 mL of phosphate-buffered solution. The incision site was sutured and animals were allowed to recover for 24 hrs before the jugular vein catheter was placed, as described in the Surgery and Experimental Protocol section of this article.

Assessment of Respiratory Mechanics

Pressure/volume loops were generated by stepwise inflations and deflations of (volume $\times$) with 1-sec breath-holds at each step. The pressure/volume points used to generate loops were those at the end of each breath-hold. The entire inflation/deflation cycle lasted 13 secs. Flexivent software (Scireq, Montreal, Quebec City, Canada) was used to calculate quasi-static pulmonary compliance and the area of the pressure/volume loops by integration.

Pulmonary resistance and elastance vary with the frequency of the applied pressure oscillation. This behavior can be modeled by a complex function of frequency called the pulmonary input impedance (Z). Respiratory impedance was measured by using the forced oscillation technique with the Flexivent system (Scireq) (21). Airway resistance (R_n), tissue damping (G), and tissue elastance (H) were obtained by fitting impedance data to the constant phase model (22). By applying a range of frequency oscillations to the tracheal opening, during a period of interrupted tidal ventilation, we are able to assess contributions to pulmonary mechanics of resistance to movement of air through airways (R), energy dissipation of lung tissue (G), and elastic properties of lung tissue (H).

Light Microscopy

Lungs were fixed in paraformaldehyde, and lung sections were stained with hematoxylin and eosin. To measure the area of the perivascular cuffs, a grid was superimposed over each micrograph, and the number of line intersections that occurred within the area between the outer edge of the smooth muscle and the nearest edge of alveolar septum was measured. The number of intersections per tissue section was averaged in each group.

Statistics

Dynamic compliance and cuff area statistics were analyzed with by one-way analysis of variance. Hemodynamic and blood gas data, perivascular loop area, quasi-static compliance, airway resistance (R_n), tissue damping (G), and tissue elastance (H) were analyzed by two-way analysis of variance with repeated measures.

RESULTS

Thapsigargin Induces Perivascular Cuff Formation Around Extra-Alveolar Vessels

Thapsigargin increases permeability in large pulmonary vessels of isolated, perfused rat and mouse lungs, but it does not increase permeability across capillary segments (6,7,10,23). In the current study we examined whether perivascular cuffs decrease the dynamic compliance by increasing tissue resistance, airway resistance, or both. Lungs from untreated rats (vehicle control dimethyl sulfoxide <0.5%) did not have perivascular cuffs (Fig. 1A). Thapsigargin injected into the internal jugular vein of ventilated, sedated rats caused perivascular cuff formation that was prominent in extra-alveolar vessels. However, as described previously in the isolated perfused rat model (6,7,10,23), histologic sections did not reveal alveolar flooding in thapsigargin treated animals (Fig. 1B and Fig. 2).

To confirm that thapsigargin did not induce alveolar edema, rats were anesthetized and jugular vein catheters were placed. Evan's blue dye albumin was infused and allowed to recirculate before delivery of vehicle or thapsigargin. After 15 mins, bronchoalveolar lavage was performed, and the presence of Evan's blue dye albumin in the bronchoalveolar lavage fluid was measured. Consistent with Figure 1, thapsigargin induced large perivascular cuffs but did not cause alveolar flooding (Fig. 3). Whereas cuffs were filled with fluid, inflammatory cells were only seen periodically and did not appear increased when compared to vehicle control animals. Thapsigargin did not increase Evan's blue dye albumin in the bronchoalveolar lavage fluid, indicating there was no increase in alveolar edema. To ensure we could measure an increase in Evan's blue dye albumin in the alveolus, we developed a *Pseudomonas aeruginosa* model of pneumonia that progresses to sepsis and acute lung injury. In this case, bacteria (5×10^7 organisms) were inoculated in the airways 24 hrs before bronchoalveolar lavage. *P. aeruginosa* caused pneumonia with significant alveolar consolidation and profound perivascular cuffs. Significant numbers of inflammatory cells, particularly neutrophils, were observed in the alveoli and in perivascular cuffs. Bronchoalveolar lavage revealed an approximate five-fold increase in Evan's blue dye

albumin in the alveoli of *P. aeruginosa*-treated animals. Hence, increased leak of Evan's blue dye albumin from the circulation into the airspace can be measured using this approach. Whereas thapsigargin does not increase alveolar fluid accumulation, *P. aeruginosa* disrupts the alveolar–capillary barrier.

To determine whether thapsigargin impacted hemodynamic parameters that could contribute to perivascular cuff formation, we monitored stroke volume, heart rate, pressure over time, left ventricular end diastolic pressure, and central venous pressures continuously in all rats (Table 1). Thapsigargin injection decreased cardiac output. Although heart rate was not reduced, stroke volume decreased significantly because of a reduction in systolic function. This was a limiting factor regarding the dose of thapsigargin that could be used and time required for drug delivery; thapsigargin delivery required a slow infusion lasting approximately 5 mins. Intracardiac peak systolic pressure generally remained >50 mm Hg during injection and recovered to systolic pressures >80 mm Hg after injection. Thapsigargin treatment did not change either left ventricular end diastolic pressure or central venous pressure. Blood gases were not generally impacted by thapsigargin treatment, although pH decreased after the infusion (Table 2). Thus, it is unlikely that decreased systolic function indirectly contributed to extra-alveolar perivascular cuff formation.

Previous reports have shown that the phosphodiesterase inhibitor, rolipram, attenuates thapsigargin-induced increases in large-vessel permeability (23,24). Interestingly, rolipram has also been shown to attenuate increased vascular permeability in rodent models attributable ischemia/reperfusion injury (25), cardiopulmonary bypass (26), and high peak inflation pressures (27). When rolipram was administered to intubated, sedated rats before administering thapsigargin, perivascular cuff formation was largely prevented (Fig. 1C and Fig. 2). Hemodynamic (Table 1) and blood gas (Table 2) parameters were not changed by the rolipram/thapsigargin treatment; rolipram prevented thapsigargin from decreasing pressure over time, stroke volume, and cardiac output. Thus, as in the isolated pump-perfused lung model, thapsigargin increases endothelial cell permeability in extra-alveolar vessels that results in perivascular cuff formation, and this effect is prevented by rolipram pretreatment.

Perivascular Cuffs Decrease Lung Compliance

Perivascular cuffs may decrease dynamic compliance by one of two mechanisms. First, perivascular cuffs may compress anatomically related bronchi and decrease airway cross sectional area, thereby increasing airway resistance (Fig. 4). A role for this mechanism is supported by studies in which extra-alveolar airways have reduced diameters because of pancreatitis-induced inflammatory changes in rats (28), in excised dog lungs with hydrostatic edema (29), and when peri-bronchial edema is induced in sheep (30). Peripheral airway resistance is also increased after an elevation in left atrial pressure and extra-alveolar edema (13–15). Second, perivascular cuffs may reduce dynamic compliance by increasing tissue resistance. Under normal circumstances, force applied at the lung periphery by the chest wall and diaphragm is transferred along alveolar septa, such that distending pressure within any portion of the lung is equal to transpulmonary pressure (Fig. 5A, B). Perivascular cuffs may induce local heterogeneity within the lung parenchyma and decrease the efficiency with which force is transferred from the periphery to the alveolar walls, thereby interrupting the coordinated expansion of the lung as a whole.

We determined the impact of perivascular cuff formation on lung compliance. Animals were ventilated using a Flexivent respirator, which enabled measurement of dynamic and static compliances. Thapsigargin reduced dynamic compliance in a dose-dependent manner (Fig. 6). Remarkably, rolipram pretreatment abolished the thapsigargin-induced decrease in lung compliance. These data suggest that perivascular cuff formation decreases dynamic

compliance, whereas rolipram pretreatment prevents perivascular cuffing and the associated decrease in dynamic compliance.

Quasi-static pressure/volume loops were generated in each rat lung. Static compliance was reduced and total area of the pressure-volume loop was increased in rats treated with thapsigargin (Fig. 7A, C) but remained unchanged in rats pre-treated with rolipram (Fig. 7B, D). To learn whether perivascular cuffs increase tissue resistance, control-treated, thapsigargin-treated, and rolipram/thapsigargin-treated rats were evaluated before and after treatment using the constant phase model. This model enables the simultaneous and independent assessment of tissue and airway resistances (22). The two components of tissue resistance, tissue damping (Fig. 8A) and tissue elastance (Fig. 8B), were both increased in lungs treated with thapsigargin and reduced by pretreatment with rolipram. These findings indicate that perivascular cuffs are sufficient to increase lung “stiffness” by increasing tissue resistance to volume expansion.

We next sought to determine whether perivascular cuff formation decreased airway resistance. Neither the thapsigargin nor the rolipram/thapsigargin treatments altered airway resistance, and nebulized albuterol had no impact on airway resistance (Fig. 9A). To ensure that increased airway resistance could be measured in our intact animal preparation, carbocholine was acutely aerosolized into the airways. Carbocholine increased airway resistance approximately three-fold, and the bronchoconstriction was acutely reversed by albuterol aerosolization (Fig. 9B). Thus, whereas perivascular cuffs are sufficient to increase tissue resistance, they do not impact on airway resistance.

DISCUSSION

During acute lung injury, the pulmonary endothelial cell barrier is disrupted, leading to extravascular fluid accumulation within the lung interstitium. Ultimately, this fluid accumulation decreases pulmonary compliance and impairs gas exchange. Traditionally, these changes have been thought to occur as the result of increased capillary permeability that leads to alveolar fluid filling and consequent surfactant inactivation (31). However, recent work illustrates that the transpulmonary endothelium is not a homogeneous cell layer, but rather one that possesses striking functional heterogeneity, particularly in regard to barrier function (32,33). Capillary endothelium forms a tighter barrier than does extra-alveolar arterial and venous endothelium (2,4). Furthermore, inflammatory and mechanical stimuli display segment-restricted actions. Stimuli such as 14,15-epoxyeicosatrienoic acid (6) and high vascular pressure (34) increase capillary permeability without increasing permeability in extra-alveolar segments. Stimuli such as thapsigargin (6,7,10,23), thrombin (35), clostridium toxin (36), and alpha-naphthylthiourea (5) increase permeability in extra-alveolar segments without increasing capillary permeability. Relatively little consideration is given to the physiologic impact of perivascular cuffs that are caused by agonists such as thapsigargin, despite evidence for cuff formation in high-altitude pulmonary edema (9) and ARDS (37,38). Here, we show that perivascular cuff formation is sufficient to produce decrements in pulmonary mechanics.

Lung injury is accompanied by impaired pulmonary mechanics, which negatively affect both the ability to mechanically ventilate critically ill patients and to wean them from the ventilator. The original ARDS criteria included a decrease in pulmonary compliance (39). Although decreased compliance has since been removed from the definition, investigations of causes of decreased pulmonary compliance during ARDS have continued. Findings of lower mortality in patients ventilated with lower volumes and lower peak pressures have highlighted the importance of considering effects of positive pressure ventilation on injured lungs (37). Our work shows that extra-alveolar endothelium possesses intrinsic signaling

pathways that, when activated, lead exclusively to large vessel leak and increased airway pressure at any given lung volume (decreased compliance). This decrease in compliance is attributable to the presence of perivascular cuffs that increase tissue resistance without increasing airway resistance. Previous studies have shown a role for both increased tissue resistance and increased airway resistance in patients with ARDS. Our present observations suggest that the perivascular cuffs that accompany ALI/ARDS contribute to the decrease in compliance observed in these patients by impairing efficient mechanical coupling between the bronchovascular bundle and the lung parenchyma.

Previous studies indicate that thapsigargin directly induces inter-endothelial cell gap formation (2,6,7,40). Our present studies support this idea, because thapsigargin induced perivascular cuffs without a concurrent increase in either central venous pressure or left ventricular end diastolic volume; it is unlikely that thapsigargin treatment increased the hydrostatic pressure in the pulmonary circulation. Nonetheless, thapsigargin treatment did impact on cardiac function, because a large decrease in cardiac output was noted. Thapsigargin inhibits the ATP-dependent sarcoplasmic, endoplasmic reticulum ATPase, which maintains high calcium concentrations within the sarcoplasmic reticulum (41). The thapsigargin-induced depletion of stored calcium activates calcium entry across the plasma membrane through so-called store-operated calcium entry channels (42). The endoplasmic reticulum ATPase calcium store is an important calcium source that modulates both cardiac excitability (43) and contractility (44). In pacemaker cells inhibition of calcium release from the sarcoplasmic reticulum reduces heart rate (45–47), whereas increasing endoplasmic reticulum ATPase expression promotes cardiac contractility (48–50). We did not observe a change in heart rate after thapsigargin infusion, although cardiac output was significantly reduced. The decrease in cardiac output occurred because of a decrease in stroke volume, as direct pressure over time measurements revealed a decrease in systolic function. It is not clear how thapsigargin decreased systolic function without changing heart rate. One explanation may be found in the recent description of store operated calcium entry in cardiac myocytes (51,52). Depletion of the sarcoplasmic reticulum calcium store may reduce actinomycin interaction and thereby decrease cardiac contractility while simultaneously activating store-operated calcium entry that is sufficient to maintain pacemaker function. Overall, our present findings are compatible with those reporting cardioprotective and inotropic actions of the endoplasmic reticulum ATPase (48–50).

Whereas the data reported suggest that perivascular cuffs impair coupling between the bronchovascular bundle and the tissue, we must consider alternate explanations that may contribute to decreased lung compliance. For example, thapsigargin may directly activate contractile machinery that increases resistance against tissue expansion, along the bronchovascular bundle and then in the microcirculation. Thapsigargin causes contraction of both pulmonary artery endothelial cells (53,54) and smooth muscle cells (55–57) in culture experiments. In the isolated perfused lung, thapsigargin infusion may cause a small, transient increase in resistance (7,58). However, in the normotensive circulation this effect is negligible, primarily because thapsigargin also induces endothelial production of nitric oxide and prostacyclin. Inhibiting nitric oxide production reveals a large, sustained, thapsigargin-induced contraction in the isolated perfused lung. The idea that thapsigargin induces endothelial cell gap formation that is accompanied by stress fiber formation while at the same time inducing nitric oxide production is very interesting and likely illustrates how calcium signals can be used to accomplish diverse physiologic outcomes. However, at present, there is no evidence that thapsigargin can stiffen the pulmonary artery vessel wall in a way that may impact tissue expansion. Thapsigargin induces pulmonary microvascular endothelial cell contraction but, by itself, does not induce gaps in these cells (23,40). This observation suggests thapsigargin could stiffen the cell. Whether such phenomena occur *in vivo* is unclear. Furthermore, it is not evident whether decreased microvascular compliance

could represent a mechanism limiting tissue expansion. Future studies would have to test this possibility. Second, we consider whether reduced cardiac contractility increases blood volume in the pulmonary circulation that impairs vascular compliance and consequently limits respiratory mechanics. In this case, rolipram treatment not only normalized perivascular cuff formation and lung mechanics but also normalized contractility. We would anticipate that if this were the cause of impaired lung mechanics, then an increase in left ventricular end diastolic pressure would accompany the decrease in contractility, or perhaps be manifest either physiologically as an increase in central venous pressure or pathologically as vascular congestion. An increase in neither central venous pressure nor vascular congestion was observed in thapsigargin-treated animals. Thus, this is an unlikely cause of limited tissue expansion, although we cannot fully exclude this explanation as a contributing factor.

CONCLUSION

In summary, by utilizing an agonist that induces perivascular cuff formation, without concomitant alveolar edema, we provide novel insight into how perivascular cuffs influence pulmonary mechanics. Our findings illustrate that perivascular cuffs that form along extra-alveolar vessels increase tissue resistance without affecting airway resistance and, in so doing, decrease lung compliance. Furthermore, we provide evidence that the type 4 phosphodiesterase inhibitor, rolipram, acutely prevents perivascular cuff formation and abnormal lung mechanics. A clinical correlate to the physiology we have described may exist in patients in whom extubation fails despite normal pre-extubation blood gases. Such patients may have perivascular cuffs that decrease their chance of successful extubation because of the high work of breathing. Future studies will determine whether prophylactic type 4 phosphodiesterase inhibition represents a novel approach to improve the success of weaning in patients at high risk for failed extubation.

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Figure 1.

Thapsigargin causes perivascular cuffing. *A*, Control animals were instrumented and vehicle control (dimethyl sulfoxide) was infused into the central vein. Lung histology reveals normal histology and an absence of perivascular fluid cuffs. *B*, Thapsigargin infusion (0.7 μM) in hemodynamically stable animals induces perivascular cuff (PVC) formation. *C*, Rolipram infusion (10 μM) before thapsigargin (0.7 μM) administration attenuates perivascular cuff formation. *A*, artery; *B*, bronchi.

Figure 2.

Thapsigargin-induced perivascular cuffs were inhibited by the type 4 phosphodiesterase inhibitor, rolipram. Cuff area was measured in histologic sections of lungs isolated from six control (97 vessels), thapsigargin-treated (125 vessels), and rolipram/thapsigargin-treated (125 vessels) animals. Six slides from each animal were examined. Grids were placed over the entire vessel, area was standardized based on magnification, and the cuff area was quantitated. Whereas thapsigargin increased total cuff area, rolipram prevented fluid accumulation. *Statistically different from control; Δstatistically different from rolipram/thapsigargin; $p < .05$.

Figure 3.

Thapsigargin induces perivascular cuffs that are largely devoid of neutrophils, without causing alveolar edema. Evan's blue dye albumin was infused through a jugular vein catheter and allowed to recirculate before delivery of vehicle control or thapsigargin. In addition, *Pseudomonas aeruginosa* (*P. aeruginosa*) was inoculated in the airways for 24 hrs, after which time jugular vein catheters were placed and Evan's blue dye albumin delivered. **A**, In control animals, perivascular cuffs are negligible in all vessels, including pulmonary arteries aligning the airways (*top left and right panels*). Thapsigargin induces significant perivascular cuffs (*middle left panel*), although these cuffs are largely devoid of inflammatory cells (*middle right panel*). Animals inoculated with *P. aeruginosa* 24 hrs previously possess large perivascular cuffs (*lower left panel*) with evidence of neutrophil accumulation (*arrows*) in the cuff (*lower right panel*). In addition, alveolar edema, alveolar hemorrhage, and consolidation were prominent histologic findings. **B**, Analysis of Evan's blue dye albumin in the bronchoalveolar lavage fluid demonstrates that thapsigargin does not disrupt the alveolar–capillary barrier. *P. aeruginosa*-treated animals display significant increases in Evan's blue dye albumin in the lavage fluid, indicating significant alveolar edema.

Figure 4.

Perivascular cuffs may impinge on airway diameter. Proposed mechanisms by which perivascular cuffs may decrease lung compliance include the possibility that bronchiole diameter is reduced. *B*, bronchi; *V*, vessel; *A*, airway.

Figure 5.

Perivascular cuffs may impact the mechanical coupling efficiency between the bronchovascular bundle and the parenchyma. *A*, Under normal circumstances, the mechanical interdependence of lung regions maintains open airways and alveoli (as shown by *arrows*). *B*, The presence of perivascular cuffs may interrupt this interdependence and allow alveolar collapse and/or increased tissue heterogeneity, leading to increased tissue resistance (as shown by *arrows*). *B*, bronchi; *V*, vessel; *A*, alveolus.

Figure 6.

Thapsigargin induces a dose-dependent decrease in dynamic compliance, which is prevented by pretreatment with rolipram. Thapsigargin was infused into the jugular vein over an approximate 5-min time course, at which time dynamic compliance measurements were performed. Thapsigargin induced a dose-dependent decrease in compliance. In separate animals, rolipram (10 μ M) was administered before thapsigargin (0.7 μ M). Rolipram pretreatment abolished the thapsigargin-induced decrease in compliance. *Statistically different from baseline ($p < .05$).

Figure 7.

Thapsigargin increases pressure-volume loop area. *A*, Representative pressure-volume curve from thapsigargin-treated rats, before and after treatment, demonstrate that thapsigargin right shifts the curve, increasing the pressure required to achieve any volume. *B*, Representative pressure-volume curve from rolipram and thapsigargin-treated rats, before and after treatment, illustrates that rolipram infusion prevents the decrease in lung compliance, normalizing the pressure-volume curve. Average data show that thapsigargin decreases static compliance (*C*) and increases the pressure-volume loop area (*D*), which are effects that are inhibited by rolipram infusion. *Different from pre-thapsigargin value ($p < .05$).

Figure 8.

Thapsigargin-induced cuff formation reduces the mechanical coupling efficiency between the bronchovascular bundle and the lung parenchyma. Impedance model in thapsigargin and rolipram and thapsigargin-treated animals. Graphs represent average values before and after treatment in each group. Both (A) tissue damping (G) and (B) tissue elastance (H) are increased in rats treated with thapsigargin. Pretreatment with rolipram reverses the thapsigargin-induced increases in G and H . *Significantly different from pretreatment control ($p < .05$).

Figure 9.

Thapsigargin treatment does not increase airway resistance. *A*, Thapsigargin infusion did not change airway resistance over a 20-min time course. Nebulized albuterol (1 mg/mL $\times$ 2 min) did not reduce baseline resistance. *Solid line* refers to control, *short dashed line* denotes thapsigargin, and *long dashed line* indicates rolipram and thapsigargin. *B*, Carbachol acutely increased airway resistance, and this effect was acutely reversed by nebulized albuterol (1 mg/mL $\times$ 2 min). * Significantly different from pretreatment ($p < .05$).

Table 1

Hemodynamic parameters measured during thapsigargin infusion reveal a decrease in contractility, stroke volume, and cardiac output

	Before Treatment			After Treatment		
	Control	Thapsigargin	Rolipram/Thapsigargin	Control	Thapsigargin	Rolipram/Thapsigargin
Cardiac output, mL	43 ± 6	42 ± 8	46 ± 9	46 ± 7	20 ± 2 ^a	52 ± 12
Heart rate, bpm	332 ± 15	328 ± 13	327 ± 16	362 ± 12	356 ± 21	346 ± 25
Stroke volume, µL	130 ± 19	128 ± 27	136 ± 21	113 ± 14	56 ± 6 ^a	99 ± 23
Contractility, mm Hg/sec	5864 ± 433	5804 ± 836	5660 ± 536	6729 ± 1017	4175 ± 357 ^a	5958 ± 1173
Left ventricular end-diastolic pressure, mm Hg	3.4 ± 1.6	4.0 ± 1.1	4.5 ± 1.5	4.5 ± 0.9	5.3 ± 1.6	4.4 ± 1.3
Central venous pressure, mm Hg	2.4 ± 0.4	1.5 ± 1.1	3.6 ± 1.4	2.9 ± 0.3	2.8 ± 0.7	4.9 ± 2.2

Cardiac output was reduced in rats treated with thapsigargin because of a decrease in contractility and stroke volume. Rolipram infusion prevented these decrements in cardiac function. Heart rate, left ventricular end-diastolic pressure, and central venous pressure were unaffected by infusions.

^aSignificantly different from control values ($p < .05$).

Table 2

Blood gases were stable throughout thapsigargin infusion

	Control		Thapsigargin		Rolipram/Thapsigargin	
	Before	After	Before	After	Before	After
P _O ₂	85 ± 3.1	89 ± 5.8	97 ± 2.5	94 ± 3.6	87 ± 2.1	85 ± 3.0
P _{CO} ₂	39 ± 1.2	40 ± 1.5	37 ± 1.2	38 ± 1.6	41 ± 3.2	45 ± 2.7
pH	7.4 ± .01	7.4 ± .01	7.4 ± .02	7.3 ± .02 ^a	7.4 ± .02	7.3 ± .02 ^a

Blood gases were equal in all groups; pH was decreased in rats treated with thapsigargin and with rolipram and thapsigargin.

^aSignificantly different from control ($p < .05$).