Continuous positive airway pressure causes lung injury in a model of sepsis

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Tsudhida, Shinya, Doreen Engelberts, Matthias Roth, Colin McKeever, Martin Post, and Brian P. Kavanagh. Continuous positive airway pressure causes lung injury in a model of sepsis. Am J Physiol Lung Cell Mol Physiol 289:L554–L564, 2005. First published May 27, 2005; doi:10.1152/ajplung.00143.2005.—Continuous positive airway pressure, aimed at preventing pulmonary atelectasis, has been used for decades to reduce lung injury in critically ill patients. In neonatal practice, it is increasingly used worldwide as a primary form of respiratory support due to its low cost and because it reduces the need for endotracheal intubation and conventional mechanical ventilation. We studied the anesthetized in vivo rat and determined the optimal circuit design for delivery of continuous positive airway pressure. We investigated the effects of continuous positive airway pressure following lipopolysaccharide administration in the anesthetized rat. Whereas neither continuous positive airway pressure nor lipopolysaccharide alone caused lung injury, continuous positive airway pressure applied following intravenous lipopolysaccharide resulted in increased microvascular permeability, elevated cytokine protein and mRNA production, and impaired static compliance. A dose-response relationship was demonstrated whereby higher levels of continuous positive airway pressure (up to 6 cmH2O) caused greater lung injury. Lung injury was attenuated by pretreatment with dexamethasone. These data demonstrate that despite optimal circuit design, continuous positive airway pressure causes significant lung injury (proportional to the airway pressure) in the setting of circulating lipopolysaccharide. Although we would currently avoid direct extrapolation of these findings to clinical practice, we believe that in the context of increasing clinical use, these data are grounds for concern and warrant further investigation.

lipopolysaccharide; spontaneous breathing; dexamethasone

CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP), designed to recruit atelectatic lung (17), is widely employed for respiratory failure (49). CPAP delivered through a face mask is useful for a spectrum of acute respiratory failure in adults (7). Such “noninvasive” CPAP support can be applied outside of intensive care units, and clinical studies have demonstrated that it may reduce the need for invasive mechanical ventilation (57, 66) and lessen the incidence of sepsis-associated complications in high-risk postoperative adult patients (57). In preterm neonates, application of CPAP, sometimes for several weeks, may reduce the need for invasive mechanical ventilation, thereby lessening complications and associated costs of neonatal care. For such neonates, a particular type of CPAP, termed “bubble” CPAP, has been developed, whereby oscillatory vibrations developed by high flow of gas through the underwater seal are transmitted to the chest. Because such a system requires minimal equipment, bubble CPAP has immense appeal in terms of operational simplicity and economic cost. Indeed, for these reasons, it has been suggested that such CPAP would be ideal for use in developing countries (27). Multiple laboratory studies over the last three decades have consistently documented the importance of lung recruitment in the prevention or abrogation of ventilator-induced lung injury (30, 41, 45, 61), where compliance is significantly impaired, or have occurred in the setting of extremes of tidal volume (63, 68). Proinflammatory cytokine production is also characteristic of pulmonary or systemic sepsis (23); in neonates, serious sepsis can be difficult to detect (60) and shares many characteristics with respiratory distress syndrome (36, 38). However, despite the potential for lung recruitment to diminish ventilator-associated lung injury (41) and reduce systemic cytokine release (9), recent laboratory data suggest that local cytokine production can be increased in the lung following surfactant administration (58), a finding consistent with regional overstretch due to increased pulmonary compliance. Thus a recruitment strategy applied in the context of evolving sepsis can negatively impact lung function and impair the ability to recruit atelectatic lungs (i.e., lungs that are not yet injured) could potentially result in either protective or injurious effects.

Because CPAP appears to be increasingly accepted as a standard neonatal intervention, we investigated whether its application would have an impact on lung injury following administration of intravenous endotoxin (LPS) in the anesthetized spontaneously breathing rat. We report that although neither CPAP nor LPS alone caused lung injury, CPAP applied following intravenous LPS caused acute lung injury as evidenced by increased microvascular permeability, cytokine protein and mRNA production, and impaired static compliance. We further demonstrated a dose-response relationship whereby higher levels of CPAP caused greater injury and, by optimizing the CPAP circuit design, confirmed that the injury was caused by the CPAP per se and not by the particular circuit used to...
administer the CPAP. Finally, pretreatment with dexamethasone (Dex) attenuated the injury.

MATERIALS AND METHODS

After approval from the Animal Care Committee of the Hospital for Sick Children (conforming to the guidelines of the Canadian Council for Animal Care), male Sprague-Dawley rats (300–450 g) were used in all experiments. General anesthesia was induced by intraperitoneal ketamine (35 mg) and xylazine (2 mg), a tracheostomy was performed, and a 14″ tracheal cannula was inserted (34). The tracheostomy tube was connected to a CPAP circuit, with a mean airway pressure of 4 cmH₂O (FiO₂ = 1.0). Arterial and venous catheters were inserted, and intravenous lactated Ringer solution was infused. Systemic arterial blood pressure, airway pressure, and temperature were continuously monitored throughout. After measurement of baseline arterial blood gas, the CPAP circuit was disconnected, and the animal breathed in an oxygen box (FiO₂ = 1.0).

CPAP delivery system. The CPAP system was composed of the oxygen gas flow, a reservoir in the inspiratory limb, a pressure transducer close to the tracheostomy connection in the expiratory limb, and a water seal (Supplementary Figure S1; Supplementary Material for this article can be found at http://ajplung.physiology.org/cgi/content/full/00143.2005/DC1), as previously described (17, 20). The level of CPAP, in cmH₂O, was adjusted by altering the depth of the water seal. An oxygen box was used for applying zero CPAP. High oxygen flow (8 l/min) was delivered to this box, and the oxygen concentration inside was ~100%.

Optimal design of CPAP circuit. To assess the effects of the CPAP circuit on the lung injury and to determine the optimal CPAP circuit design (i.e., to ensure that circuit design did not cause lung injury), we compared the following three different types of CPAP circuit: low flow with a reservoir (LF-R), high flow with a reservoir (HF-R), and high flow without a reservoir (HF-R).

The CPAP level was 4 cmH₂O and FiO₂ was 1.0 in all groups. In the LF+R group, the oxygen gas flow was adjusted to maintain a steady egress of bubbles from the end of the CPAP circuit (~0.5 l/min). In contrast, high oxygen flow was 6 l/min, which resulted in visible vibration of the chest. Pilot experiments had demonstrated that CPAP alone was not injurious but was injurious following administration of LPS (data not shown). Therefore, to assess the effects of different circuit constructs, saline only (i.e., no LPS) was administered. Following the animal preparation as before, the animals received intravenous injection of 0.9% saline (12 ml/kg) and were allocated to one of the following circuits: LF+R, HF+R, or HF-R. After the allocation, anesthesia was maintained with intravenous ketamine and xylazine. Mean arterial blood pressure, airway pressure, respiratory rate, and arterial blood gas were recorded every 30 min. Evans blue dye (20 mg/kg; Sigma, St. Louis, MO) was administered intravenously to all animals 30 min after the allocation (34). After 3 h, animals were exsanguinated by opening the arterial line, and the lung-heart block was removed. Evans blue dye (20 mg/kg) was administered intravenously to all animals 30 min after the allocation of CPAP (34/CPAP-2), CPAP with 4 cmH₂O (CPAP-4), and CPAP with 6 cmH₂O (CPAP-6). The CPAP circuit of LF+R was adopted in this study. The oxygen inflow was adjusted to maintain bubble egress from the CPAP circuit. Evans blue dye (20 mg/kg) was administered intravenously to all animals 30 min after the allocation. After CPAP for 3 h, the lung injury was assessed by measurement of CPAP-2, LPS/CPAP-2, CPAP with 4 cmH₂O (CPAP-4), and CPAP with 6 cmH₂O (CPAP-6). The CPAP circuit of LF+R was adopted in this study. The oxygen inflow was adjusted to maintain bubble egress from the CPAP circuit. Evans blue dye (20 mg/kg) was administered intravenously to all animals 30 min after the allocation. After CPAP for 3 h, the lung injury was assessed by measurement of CPAP-6. The CPAP circuit of LF+R was employed in this study. The oxygen inflow was adjusted to maintain bubble egress from the CPAP circuit (0.5 l/min). Following the animal preparation as before, animals were allocated to one of four groups (Supplementary Figure S2). Animals initially received either intraperitoneal 10 mg/kg Dex (Saxbe, Quebec, Canada) or an equivalent volume 0.9% saline 3 h before animal preparation. Following animal preparation as before, all animals received intravenous injection of 30 mg/kg LPS (lot no. 113K0487), were exposed to CPAP (4 cmH₂O), and then were observed for 3 h. Lung injury was assessed as above. After measurement for Evans blue absorbance, BAL samples were stored at −70°C for cytokine protein concentrations.
BAL cytokine measurements. The appropriate cytokine standards and BAL samples (25 μl) diluted in the same volume of assay buffer (PBS, 1% BSA, and 0.08% sodium azide) were added to wells of a filter plate preequilibrated with assay buffer. The samples were incubated with 25 μl of the antibody-coupled beads selected for the assay on a plate shaker overnight at 2–8°C. Twenty-five microliters of detection antibody cocktail were added to the wells and then incubated on a plate shaker for 2 hours at room temperature. Twenty-five microliters of streptavidin-phycoerythrin were added to the wells, and the incubation at room temperature continued for 30 min. The unbound fraction was filtered through the wells using the vacuum manifold, and the bound beads were washed twice with 200 μl/well of wash buffer. After the last wash step, 100 μl of sheath fluid were added to each well, and the plate was placed for 5 min on a plate shaker. The plate was run on Luminex™ and analyzed according to the manufacturer’s instructions (RCYTO, Linco Research) (53, 67).

RNA isolation and real-time PCR. The frozen left lung tissue was homogenized in TRIzol (Invitrogen), and total RNA was extracted using chloroform and isopropanol. After being washed, the RNA was incubated with DNase I (Invitrogen) at 37°C and eluted. The RNA (2 μg) was reverse transcribed in a total volume of 20 μl using Super-Script II reverse transcriptase (Invitrogen) and random primers. cDNA was further amplified in a 7700 Sequence Detector (Applied Biosystems, Foster City, CA) using 100 ng of cDNA for IL-6, IL-1β, and TNF-α and 40 ng of cDNA for macrophage inflammatory protein-2 (MIP-2). The amplification was performed with Amply Tag Gold polymerase (Applied Biosystems) using TaqMan primers and probes as recently described (11). For the relative quantification, PCR signals were compared among groups after normalization using 18S as an internal reference. Fold change was calculated according to Livak and Schmittgen (39).

Statistics. Statistical analysis was performed as previously recommended (13). The data are expressed as means ± SD. For among-group comparisons in all the multigroup experiments, ANOVA was used followed by Student-Newman-Keuls tests. For the single two-group experiments (LPS + CPAP + Dex vs. LPS + CPAP-Dex), unpaired Student’s t-test was used. The correlation coefficient was calculated using linear regression. Significance was set at P < 0.05.
RESULTS

Optimal design of CPAP circuit. Eighteen rats were utilized in this series, and all animals survived the entire protocol. All baseline parameters (i.e., animal weight, mean arterial blood pressure, and blood gas data) were comparable in the three groups throughout the 3 h (Supplementary Table S1). The LF/HF circuit was not associated with demonstrable injury; however, the other two circuits were, and the rank order of lung injury, indicated by pulmonary vascular leak, wet/dry ratio, and impairment of static compliance, was LF/HF > HF/HF-R (P < 0.05, Fig. 1, A–C).

Effects of LPS and CPAP. Having determined the optimal (i.e., least injurious) CPAP circuit, we then investigated the interactions of LPS (iv LPS vs. iv saline) and CPAP (CPAP-4 cmH2O vs. no CPAP) in the following four experimental groups: saline-CPAP (without CPAP), saline+CPAP (with CPAP), LPS-CPAP (without CPAP), and LPS+CPAP (with CPAP). Eight animals died following LPS administration and were replaced; thus 32 rats were ultimately allocated to CPAP or no CPAP (Supplementary Figure S2). One animal died in the LPS-CPAP group, and one died in the LPS+CPAP group. All other animals completed the experiment. All baseline parameters (i.e., animal weight, mean arterial blood pressure, arterial pH, PaO2, PaCO2, HCO3−, and base excess) were comparable in the four groups (Supplementary Table S2). Compared with the saline-treated animals, LPS-treated animals demonstrated lower PaCO2 at 180 min (P < 0.05). At the conclusion of the study, there was no difference in the PaO2 among the four groups (Supplementary Table S2).

The lung injury assessed by microvascular leak and wet/dry ratio was significantly higher in LPS+CPAP compared with the other three groups (i.e., LPS-CPAP, saline+CPAP, saline-CPAP; P < 0.05, Fig. 2, A and B). The static P-V curve

Fig. 3. Pressure-volume (P-V) curves following the completion of the study. The 4 symbols represent the averaged data from the P-V curves in the following groups: saline-CPAP (○), saline+CPAP (■), LPS-CPAP (●), and LPS+CPAP (▲). Static compliance was lowest in LPS+CPAP (SD are omitted from this figure for clarity. See Supplementary Figure S3 for full details).

Fig. 4. Effects of LPS and CPAP on the cytokine protein concentrations in bronchoalveolar lavage (BAL). A: animals in LPS-CPAP showed higher levels of IL-6 vs. saline-treated animals (†P < 0.05). The combination of CPAP with LPS resulted in much higher levels of IL-6 vs. the other 3 groups (*P < 0.05). B: IL-1β protein concentration was higher in LPS+CPAP vs. saline-treated animals (†P < 0.05). C: TNF-α protein concentration was higher in LPS+CPAP than the other 3 groups (*P < 0.05). All data are means ± SD.
showed that the static compliance was most impaired in the LPS + CPAP group (Fig. 3 and Supplementary Figure S3).

BAL fluid was assayed for the following cytokine proteins: IL-6, IL-1β, and TNF-α. IL-6 was almost negligible in the saline-treated animals regardless of whether or not CPAP was applied (Fig. 4A). LPS without CPAP resulted in slightly, but significantly, higher levels of IL-6 \((P < 0.05, \text{Fig. 4A})\). However, the combination of CPAP with LPS resulted in far greater levels of IL-6 in the BAL \((P < 0.05)\), suggesting a synergistic effect of LPS and CPAP (Fig. 4A). Compared with the saline-treated animals, IL-1β was significantly higher in LPS + CPAP \((P < 0.05)\), but there was no significant difference between LPS-CPAP and LPS + CPAP (Fig. 4B). LPS in the absence of CPAP did not impact on the BAL TNF-α protein compared with the saline-treated animals, but LPS in the presence of CPAP caused a marked increase in the BAL TNF-α protein, resulting in a significant difference compared with the other three groups \((P < 0.05, \text{Fig. 4C})\).

Quantitative PCR was performed on the dependent and nondependent tissues collected from four animals in each group plus four control animals (not exposed to LPS or to CPAP). They were assayed for the mRNA expression of the following cytokines: IL-6, IL-1β, MIP-2, and TNF-α. The levels of expression of cytokine mRNA did not differ between dependent vs. nondependent lung regions in any of the groups (data not shown). Therefore, the data of dependent and non-dependent tissue were averaged and expressed as the fold change of mRNA for each animal. The mRNA expression of all four cytokines was higher in LPS + CPAP vs. control lungs \((P < 0.05)\), with the values for saline + CPAP, saline-CPAP, and LPS-CPAP at intermediate levels (Fig. 5, A–D).

Representative microscopic findings of lung tissue stained with hematoxylin-eosin (×200 magnification) are shown in Fig. 6. Saline-treated animals showed intact alveolar walls and histologically normal air spaces (Fig. 6, A and B). A section from LPS-CPAP showed minimal polymorphonuclear infiltration within the alveolar walls, but with little edema or free-floating intra-alveolar macrophages and negligible-to-mild overall lung injury (Fig. 6C). A section from LPS + CPAP showed moderate thickening of alveolar walls with significant polymorphonuclear infiltration, intra-alveolar edema with inflammatory cell exudates, and hypertrophy of alveolar epithelial cells, all of which are consistent with diffuse alveolar damage (Fig. 6D).

Dose-response characteristics of CPAP. The effects of LPS in conjunction with several levels of CPAP (0–6 cmH2O) were studied. Twelve additional rats were utilized in this series, and eight survived the entire protocol. Blood gas and acid-base
parameters were comparable in the four groups throughout the 3 h (Supplementary Tables S2 and S3). There was a strong correlation between the level of CPAP and pulmonary vascular leak, wet/dry ratio, and degree of impairment of static compliance (Fig. 7, A–C).

Pretreatment with Dex. In these experiments, one animal died following the LPS administration and was replaced; thus 12 rats were exposed to CPAP (4 cmH2O). No animal died in the Dex group, but one animal died in the saline group. The mean systemic blood pressure was higher in the Dex group throughout (Supplementary Table S4). There was no difference in the PaO2, but animals showed lower PaCO2 values in the saline group after 2 h (Supplementary Table S4, P < 0.05).

The lung injury assessed by microvascular leak, lung wet/dry ratio, and impairment of static compliance was significantly less in the Dex group compared with the saline group (P < 0.05), confirming that pretreatment with Dex attenuated the lung injury (Fig. 8, A–C). BAL fluid was assayed for IL-6 and TNF-α protein (the significantly altered cytokines from the previous experiments), and pretreatment with Dex (vs. saline) was associated with significantly lower levels of IL-6 and TNF-α protein (Fig. 9, A and B).

DISCUSSION

The current study demonstrates that in this model of sepsis (i.e., circulating LPS) and a normally compliant respiratory system, application of CPAP is not beneficial; instead, it causes lung injury. These data are of concern because they suggest that whereas recruitment of the injured lung may prevent or reduce lung injury, recruitment of noninjured (i.e., compliant) lung, in the setting of circulating LPS, may cause harm. Only the lungs of rats that were exposed to both CPAP and LPS were analyzed. However, the effects of CPAP, compared with conventional mechanical ventilation, in reducing acute lung injury in preterm lambs (24) and preservation of normal alveolar development in preterm primates (62), benefit has been reported in the setting of ischemia-reperfusion in adult experimental models (14). In the clinical context, CPAP has been widely accepted as a means of respiratory support since its initial description by Gregory et al. (17) as a means of avoiding endotracheal intubation in preterm neonates (66) as well as adults (51). Indeed, a more recent comparison between two tertiary care neonatal centers suggested that, compared with conventional mechanical ventilation, the primary use of CPAP was associated with a lower incidence of bronchopulmonary dysplasia (65), a major cause of morbidity among neonatal intensive care survivors. Finally, current estimates suggest that CPAP is used in approximately one-half (and, especially in northern Europe, the majority) of preterm infants worldwide (18, 28). Therefore, the compelling rationale for using CPAP is matched by strong evidence of its widespread use in clinical practice.

However, clinical concerns extend beyond injury and development of the lung, and whereas sepsis is the most common cause of death in adults with lung injury (16), it is also an important cause of neonatal mortality (40). Indeed, in neonates, it is often difficult to distinguish sepsis from the respiratory distress syndrome (60). This problem can be confounded by the diagnostic difficulties of “culture-negative” sepsis as well as concurrent antibiotic use (47). Thus CPAP may well be applied in the setting of evolving, but unrecognized, sepsis. Unfortunately, although the benefits of CPAP in nonseptic respiratory failure seem apparent (17, 24, 57, 62, 65, 66), its effects in evolving sepsis or endotoxemia have not been reported.

Recruitment, lung injury, and sepsis. Outcome studies demonstrate that sepsis is an important cause of lung injury in...
adults (16) as well as in neonates (22). In addition, the prospective demonstration that mechanical ventilation can have an impact on outcome following lung injury (4), coupled with the recent laboratory findings that high tidal volume ventilation potentiates lung injury following LPS administration (2, 6), suggests that sepsis may synergistically interact with adverse ventilation (or more simply, overdistension) to produce adverse outcome. Indeed, animal models of adult (44) and neonatal (64) lung injury indicate that adverse patterns of mechanical ventilation, high tidal volume, and absence of recruitment function as a “second hit” to previously “primed” lungs.

In contrast to the adverse effects of such “nonprotective” ventilation, maintenance of lung volume has been associated with lung protection in laboratory studies of ventilator-induced lung injury (41, 68) and reperfusion injury (14). Indeed, a clinical study suggests that ensuring lung recruitment through “prophylactic” CPAP may lessen the need for mechanical ventilation, as well as reduce the development of multiple organ dysfunction and sepsis, in high-risk postoperative adult

Fig. 7. Dose-response relationship between the CPAP level and lung injury in animals pretreated with LPS. A: correlation between level of CPAP and microvascular leak (BAL absorbance; \( r = 0.812, P < 0.001 \)). B: correlation between level of CPAP and wet/dry ratio \( (r = 0.877, P < 0.001) \). C: correlation between level of CPAP and total lung capacity \( (r = -0.571, P < 0.005) \). The following 4 groups were compared, all pretreated with LPS: zero CPAP (CPAP-0), CPAP with 2 cmH\(_2\)O (CPAP-2), CPAP with 4 cmH\(_2\)O (CPAP-4), and CPAP with 6 cmH\(_2\)O (CPAP-6).

Fig. 8. Effects of pretreatment with dexamethasone (Dex) on pulmonary microvascular leak and lung static compliance. Animals in both groups received 30 mg/kg LPS and 4 cmH\(_2\)O CPAP. A: microvascular leak was lower in Dex group than saline group \( (*P < 0.05) \). B: wet/dry ratio was lower in Dex group than saline group \( (*P < 0.05) \). C: total lung capacity was higher in Dex group than saline group \( (*P < 0.05) \). All data are means ± SD.
patients (57). These positive effects of recruitment have parallels in the neonatal setting, where CPAP is associated with a lower expression of IL-6 mRNA than conventional, albeit “gentle” mechanical ventilation in the preterm lamb (43).

However, recruitment of lung may not always be beneficial. Randomized, controlled trials of positive end-expiratory pressure (PEEP) as prophylaxis against (50) or during treatment (8) for acute respiratory distress syndrome did not demonstrate a benefit. In uninjured isolated mouse lungs, administration of surfactant resulted in increased levels of proinflammatory mediators (e.g., IL-6 and TNF-α), consistent with greater distension afforded by the surfactant-induced increase in lung compliance (58). Earlier studies demonstrated similar effects of overdistension associated with surfactant in the in vivo lamb (42, 46). In these studies, higher levels of PEEP (i.e., 7 cmH₂O) were associated with higher static compliance and also earlier death in spontaneously breathing lambs (37). Together with two studies of lung distension (37, 58), the current findings suggest that in highly compliant lungs, CPAP acts as a second hit and can potentiate an evolving inflammatory process and, in this case, the pulmonary responses to LPS. The increased cytokine production is attributed to the synergy of LPS and CPAP-induced overdistension, with the possibility that the proinflammatory mediator release might have contributed to the ongoing injury.

Although the tissue injury responses were worse with CPAP following the administration of LPS, the final Pao₂ level was comparable among the four groups. If translated to the clinical setting, this would be an additional concern because CPAP can protect against deoxygenation the same way PEEP does, and CPAP could mask evolving lung injury by maintaining excellent systemic oxygenation.

Choice of CPAP circuit. There are two major types of CPAP devices, demand or continuous flow systems. The demand systems are usually incorporated in the complex intensive care ventilators, whereas the continuous flow devices are not only cheaper but may also be more efficient. The continuous flow devices can utilize either high gas flow exceeding the peak inspiratory flow rate or low gas flow in conjunction with a reservoir (21). In any CPAP circuit, potential gas flow must exceed the patient’s peak inspiratory flow; thus, if low gas flow is used, the circuit must incorporate a reservoir (20). Although theoretically advantageous, variable-flow systems actually offer no advantages in terms of work of breathing (12) and may be associated with pulmonary hyperinflation (48).

The current study tested three different models of CPAP circuit in the absence of LPS and then employed that design associated with the least degree of lung injury (i.e., low gas flow plus presence of a reservoir: LF+R) in the subsequent detailed analysis of the interaction between CPAP and LPS. In fact, such a design has previously been shown to be associated with the least work of breathing and most stable airway pressure (20). In addition, the observed lung injury in the high flow groups may be related to the forced insufflation of oxygen through the entire tracheal cannula during the expiratory phase, because such an insufflation may give rise to an unexpected increase of intrapulmonary pressure and lung volume, which cannot be routinely monitored (26). The current study suggests that in the presence of LPS, alternative circuit designs would be associated with even greater degrees of lung injury; specifically, higher flow rates were associated with lung injury in the current study.

The concept of bubble CPAP involves the visible vibration of chest, and in this context, high gas flow is mandatory for such effects. The chest vibrations are similar to the waveforms produced by high-frequency ventilation and associated with a decrease in respiratory rate in preterm neonates (35). However, the effects of chest vibration on lung injury are unknown, although in the context of high-frequency oscillation, there is probably no harm, and likely benefit (41). In the current study, chest vibration was observed with both forms of high flow, but not with low flow.

Level of CPAP. The level of CPAP chosen (4 cmH₂O) for detailed analysis was the midrange of the tested levels of CPAP (2–6 cmH₂O), where there was a stepwise “dose response” demonstrated between the level of applied CPAP, administered as cmH₂O, and the degree of lung injury. Indeed, CPAP of 4 cmH₂O represents a clinically relevant level used in newborn infants (15, 25) and is less than the usual levels employed in adults (49, 51).

Mechanisms of injury and cytokine release. There are multiple possible mechanisms of the interactions between LPS and...
CPAP observed in the present study. First, LPS is known to initiate transcription of proinflammatory mediators via activation of the membrane-bound Toll-like receptor-4 (Tlr-4), which, when activated, initiates translocation of NF-kB into the nucleus (5, 52, 56). Such NF-kB translocation is also elicited by ventilatory stretch, resulting in the release of proinflammatory cytokines that appear to contribute to the pathogenesis of ventilator-induced lung injury (19). The activation of NF-kB is associated with degradation of IκBα, the transcription of which is upregulated by Dex (54, 55). In addition, Dex has multiple concomitant anti-inflammatory actions [e.g., downregulation of IL-6, IL-8, regulated upon activation, normal T cell expressed and secreted (RANTES)] that could also contribute to the effects observed in this report (1). The chemokine RANTES is a potent chemoattractant for eosinophils, lymphocytes, and monocytes. RANTES has been detected in the epithelium of human airway mucosa and can be downregulated by glucocorticoids (59). Although the effects of specific blockade of Tlr-4 receptors or IκBα degradation were not specifically examined in the current study, the LPS/CPAP-induced increase in cytokine mRNA that accompanied the elevation in cytokine proteins (TNF-α, IL-1β, IL-6) suggests that the injury may have been mediated via such a transcription-activation mechanism; such a mechanism is supported by the fact that pretreatment with Dex attenuated the lung injury. Indeed, previous work from our group suggests such a mechanism (in the absence of LPS but with high tidal lung stretch) and further indicates that the site of such activation likely resides in the bronchial epithelium (10). Second, the use of an in vivo model, while conferring a greater degree of clinical relevance, invariably involves multiple additional secondary events (e.g., hypotension, acidosis), which may contribute to the pathogenesis of the observed injury. We avoided fluid resuscitation and do not know whether such resuscitation would have attenuated or worsened the observed injury. Nonetheless, although individual dissection of such effects is not possible in vivo, the model serves to illustrate the integrative biological effects resulting from application of a technique to an intact animal. Indeed, although stretch and LPS may have some signaling pathways in common (19), they clearly represent very different stimuli. The current in vivo study demonstrates clearly that although the dose of LPS was sufficient to cause significant hypotension and was lethal in 30% of cases, it caused no demonstrable lung injury (i.e., minimal cytokine production or changes in pulmonary physiological parameters) in the absence of CPAP. Such a picture would be unlikely in the presence of injurious lung stretch alone, where lung injury would be expected to appear before changes in systemic physiology (69). Third, pretreatment with Dex was examined because of the previously identified effect of corticosteroid on stretch-induced lung injury (19). Although insufficient to elucidate the specific pathways, the attenuation of injury by Dex supports an inflammatory basis for the injury. Finally, Mulrooney et al. (43) examined the effects of different levels of CPAP in preterm (i.e., surfactant-deficient) neonatal lambs and demonstrated that without exogenous surfactant, higher levels of CPAP resulted in greater surfactant function. When exogenous surfactant was administered to the preterm lambs, lung recruitment with a higher level of PEEP was associated with better surfactant function, but greater protein leakage and morphological overdistension (42, 46). We did not examine surfactant function in the current study. However, it is possible that CPAP-induced increases in lung surfactant permitted greater inflation in these mature, surfactant-replete lungs, with high levels of chest wall compliance, which instead of being beneficial, resulted in injury from gross overdistension, as has been demonstrated with the addition of surfactant in an ex vivo model (58).

Study limitations. In the current study, we used in vivo rats spontaneously breathing under general anesthesia. Animals in the LPS+CPAP group developed significant hypotension and acidosis, possibly contributed to by impeded venous return. However, the inclusion of saline-treated controls indicates that these effects were due not to the CPAP per se but rather to an interaction of CPAP and the LPS. Unlike isolated organ systems, here, as in any in vivo model, clear differentiation of systemic vs. pulmonary events is not possible; of course, restriction to an isolated lung system would have an alternative set of inherent limitations. The circuit used in the current study is similar to that used in bubble CPAP; however, there are many differences between the clinical practice of bubble CPAP and our experimental CPAP setting. First, bubble CPAP is clinically used in neonates, not in adults. Bubble CPAP has been studied in the neonates of larger animal species (24, 43, 62), but its feasibility in neonatal rats or mice has yet to be demonstrated. Second, bubble CPAP is usually given nasally to the neonates, whereas CPAP was given endotracheally to the animals in this study. Indeed, previous authors have speculated that airway resistance may be greater with CPAP applied to neonates via tracheostomy compared with the nasal route (31, 32); however, the current study suggests that because the higher level of CPAP caused progressively greater lung injury, increased inspiratory airway resistance was not a limitation of the tracheostomy in these experimental animals. Finally, animals breathed nonhumidified 100% oxygen, which is seldom used clinically. However, given the short duration of the experimental protocols, this is not likely to represent a serious limitation.

Conclusions. The current data demonstrate that CPAP, in the context of systemic endotoxin, causes significant lung injury that increases with increased levels of CPAP, is not explained by circuit design but is attenuated by pretreatment with Dex. We would avoid direct extrapolation of the current findings to the clinical setting because the rats were healthy adult animals, nonhumidified 100% oxygen was used, and the dose of Dex was high. Nevertheless, we believe that the data are grounds for concern and warrant further investigation.

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