Influence of Dexamethasone on Protease-Activated Receptor 2-Mediated Responses in the Airways

Sham Mohd Saleh, Tracy S. Mann, Terence Peters, Richard J. Betts, and Peter J. Henry
Pharmacology and Anaesthesiology Unit, School of Medicine and Pharmacology, University of Western Australia, Nedlands, Australia

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ABSTRACT

Stimulants of protease-activated receptor (PAR)2 promote the generation of the bronchoprotective prostaglandin (PG) E2 by airway epithelial cells. In contrast, glucocorticoids reduce the levels of PGE2 in airway epithelial cell cultures by concomitantly inhibiting pathways required for PGE2 synthesis and facilitating pathways involved in PGE2 inactivation. The aim of this study was to determine whether glucocorticoids inhibited PAR2-mediated, PGE2-dependent responses in epithelial cell cultures, in intact airway preparations, and in whole animals. In cultures of A549 cells, a PAR2-activating peptide SLIGRL-NH2 produced concentration and time-dependent increases in PGE2 levels, which were significantly enhanced after exposure to lipopolysaccharide (LPS). However, SLIGRL-NH2-induced increases in PGE2 levels were abolished by pretreatment of cells with the glucocorticoid, dexamethasone. In mouse isolated tracheal preparations, SLIGRL-NH2 and PGE2 induced concentration-dependent relaxation responses that were unaffected by dexamethasone, irrespective of whether dexamethasone exposure occurred in vitro or in vivo. Intranasal administration of LPS produced a pronounced increase in the numbers of neutrophils recovered from the bronchoalveolar lavage fluid of BALB/c mice. Numbers of recovered neutrophils were 40 to 60% lower in mice that received f-LIGRL-NH2 (PAR2-activating peptide, 30 μg intranasally), PGE2 (10 μg intranasally), or dexamethasone (1 mg/kg i.p.). In the combined presence of dexamethasone and f-LIGRL-NH2 or dexamethasone and PGE2, the number of neutrophils was suppressed further (80–83% lower). Thus, although dexamethasone abolished PAR2-mediated generation of PGE2 in A549 cells, neither the smooth muscle relaxant nor the anti-inflammatory effects of PAR2-activating peptides (and PGE2) were diminished by in vitro or in vivo exposure to dexamethasone.

Protease-activated receptor (PAR) 2 is abundantly expressed by bronchial smooth muscle and epithelial cells (Schmidlin et al., 2001). Activation of epithelial PAR2 promotes the generation and release of a variety of cytokines and mediators, including prostaglandin (PG) E2 (Lan et al., 2001; Asokananthan et al., 2002; Kawabata et al., 2004a). PAR2-mediated production of PGE2 occurs via the nontranscriptional activation of enzymes involved in PGE2 synthesis, including cyclooxygenase (COX) and prostaglandin E synthase (PGES) (Kawabata et al., 2004a). Of particular interest, PAR2 is one of only a small number of receptor subtypes that have been shown to be linked to the rapid generation of epithelial PGE2, which has many beneficial actions in the airways (for review, see Vancheri et al., 2004). Epithelial-derived PGE2 is released toward submucosal structures (Perng et al., 2003), where it can activate prostanoid (EP) receptors and produce a range of beneficial effects, including bronchodilatation and suppression of the function of mast cells, eosinophils, and fibroblasts. Consistent with this, exogenous application of PGE2 inhibited allergen-induced inflammation and bronchoconstriction in human asthmatic subjects (Gauvreau et al., 1999) and in animal models of allergic airways inflammation (De Campo and Henry, 2005). Likewise, strategies that suppress the production of PGE2, such as the administration of drugs that inhibit COX and the use of COX−/− mice, were associated with worsening allergic inflammation (Carey et al., 2003) and fibrotic disease (Hodges et al., 2004). PAR2 activators such as SLIGRL-NH2 produced bronchoprotective effects in a murine model of allergic inflammation via COX-dependent mechanisms (De Campo and Henry, 2005).

Glucocorticoids are used widely to suppress airways inflammation in people with persistent asthma and are likely to continue to be the cornerstone of asthma therapy for the foreseeable future. Indeed, the central importance of glucocorticoids in the management of persistent asthma is highlighted by the fact that other pharmacotherapies, such as...
long-acting β-adrenoceptor agonists and leukotriene receptor antagonists, are typically coadministered with glucocorticoids rather than used alone. Glucocorticoids have been shown to significantly reduce PGE₂ levels in airway cells via a range of genomic and nongenomic actions; for example, by inhibiting the expression of PGES and COX (Mitchell et al., 1994; Newton et al., 1997; Chivers et al., 2004) and suppressing the release of arachidonic acid, an initial substrate in the synthesis of PGE₂ (Chivers et al., 2004). Glucocorticoids may also reduce the levels of PGE₂ by increasing the expression of prostaglandin dehydrogenase, the principal enzyme responsible for metabolizing PGE₂ (Tong and Tai, 2005). Consistent with this, it has been shown that neutrophil elastase-induced production of PGE₂ in human airway epithelial cells, perhaps mediated via PAR₂, was abolished by glucocorticoids (Perring et al., 2003). However, glucocorticoid-induced suppression of PGE₂ levels has been observed almost exclusively in cell culture systems, which may not directly reflect the situation in intact tissue and whole animal systems. Thus, the aim of the current study was to determine whether potential bronchoprotective effects produced by PAR₂ activators, such as PGE₂ production, airway smooth muscle relaxation, and anti-inflammatory effects were modulated in cells, tissues, and animals exposed to glucocorticoids. A series of studies were conducted in cultured A549 cells, cultured mouse tracheal segments, or BALB/c mice that had been pretreated with dexamethasone (in the presence and absence of a proinflammatory stimulus lipopolysaccharide or saline vehicle) for 24 h, washed in serum-free medium overnight, and then exposed to combinations of selected agents in serum-free medium. In initial experiments, A549 cells were exposed to the PAR₂-activating peptide SLIGRL-NH₂ (or a partially scrambled control peptide, LSIGRL) at different concentrations (1–100 M) for different periods of time (0.25–24 h), and the medium was collected and stored at −80 °C for subsequent determination of PGE₂ levels. In other experiments, cells were preincubated with dexamethasone (1 μM or cyclodextrin vehicle) in the presence or absence of a proinflammatory stimulus (10 μM lipopolysaccharide or saline vehicle) for 24 h, washed in serum-free medium, and stimulated with 50 μM SLIGRL-NH₂ for a further 30 min, at which time the medium was collected and stored for PGE₂ assay. Aliquots of medium were assayed for PGE₂ using a commercial enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) following the manufacturer's instructions.

Isometric Tension Recording Studies Using Mouse Isolated Tracheal Segments. Male BALB/c mice (specific pathogen free) at 8 to 10 weeks of age (Animal Resources Centre, Perth, Australia) were maintained in a 12-h light-dark cycle and received autoclaved rodent food pellets and acidified water ad libitum. All studies were conducted with the approval of the University of Western Australia’s Animal Ethics Committee. Mice were killed by lethal injection (250 mg/kg pentobarbitone sodium i.p.), and the trachea were harvested.

Segments of mouse trachea were suspended between two stainless steel hooks at a resting tension of 0.4 g in a tissue bath containing 2 ml of Krebs-bicarbonate solution, maintained at 37°C, and bubbled with 5% CO₂ in O₂. The composition of Krebs-bicarbonate solution was: 117 mM NaCl, 5.36 mM KCl, 25 mM NaHCO₃, 1.03 mM KH₂PO₄, 0.57 mM MgSO₄·7H₂O, 2.5 mM CaCl₂, and 11.1 mM d-glucose. The lower hook was attached to a micrometer driven anchor in the bath, and the upper hook was attached to a force-displacement transducer (model FT03C; Grass Instruments, West Warwick, RI) coupled to a PowerLab system (ADInstruments Pty Ltd., Bella Vista, Australia). Tracheal preparations were washed regularly for 30 min, and the tension was periodically readjusted to 0.4 g. On two consecutive occasions separated by a 15-min washout period, preparations were exposed to cumulatively administered doses of carbachol (0.2 and 10 M). After a carbachol 20-min washout period, a cumulative concentration-response curve was constructed for carbachol (0.03–10 M). To assess PAR₂-mediated relaxation responses, tracheal preparations were precontracted with 1 μM carbachol (producing approximately 60% of the contractile response obtained by 10 μM carbachol), and upon reaching a plateau level of contraction, a single 2 μM concentration of SLIGRL-NH₂ was added for 5 min, during which time a peak relaxation response was obtained. The preparation was washed with drug-free Krebs-bicarbonate solution and re-equilibrated for 20 min before the cycle was repeated a further three times to determine the relaxant responses to 5, 10, and 20 μM SLIGRL-NH₂. A cumulative concentration-response curve was then constructed to exogenous PGE₂ (3–3000 nM) in preparations also precontracted with 1 μM carbachol. In these studies, tissues were exposed to 1 μM dexamethasone (or cyclodextrin vehicle) for 15 min before each carbachol-induced contraction and retained in the bath until the response to carbachol, SLIGRL-NH₂, or PGE₂ had been obtained (short-term in vitro dexamethasone exposure). In all cases, the level of relaxation response was calculated as a percentage of the level of preconstriction, such that 100% relaxation represents a complete reversal of the carbachol-induced precontraction.

Tissue Culture Studies Using Mouse Isolated Tracheal Segments. In a separate series of experiments, mouse isolated tracheal segments were cultured using the method of Bachar et al. (2005). In brief, tracheal segments obtained from BALB/c mice as described above were cultured in Dulbecco’s modified Eagle’s medium, supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, at 37°C with 5% CO₂/95% air for 3 days. During the 3 days of organ culture, tracheal segments were exposed to dexamethasone (1 μM, or cyclodextrin vehicle) and transferred daily to a new well containing fresh media and dexamethasone (long-term in vitro exposure to dexamethasone). After organ culture, tracheal segments were transferred to tissue baths and isometric tension recording studies completed, as described above.

Whole-Animal Studies Using BALB/c Mice. As an extension of the studies described above in which tracheal segments were exposed to dexamethasone in vitro, male BALB/c mice were given dexamethasone in vivo (3 mg/kg or cyclodextrin vehicle i.p.). Twenty-four hours later, mice were sacrificed, and their trachea were removed and used in isometric tension recording studies (in vivo exposure to dexamethasone). The influence of in vivo pretreatment with dexamethasone (3 mg/kg or cyclodextrin vehicle i.p.) on subsequent in vitro responsiveness to carbachol, SLIGRL-NH₂, and PGE₂ was also determined in mice that were exposed to the proinflammatory stimulus lipopolysaccharide at the time of dexamethasone administration. Lipopolysaccharide (10 μg in 20 μl of sterile saline) was administered intranasally by applying the aliquot to the external nares of mice that had been anesthetized with methoxyflurane.

PAR₂ activators such as SLIGRL-NH₂ have previously been reported to inhibit bacterial lipopolysaccharide-induced recruitment of polymorphonuclear leukocytes into the Airways of BALB/c mice (Moffatt et al., 2002). A final series of studies was conducted to determine whether this effect was modulated by dexamethasone. Mice were pretreated for 3 days with daily injections of dexamethasone (1 mg/kg or cyclodextrin vehicle i.p.), and then inoculated intranasally with a 20-μl solution containing combinations of lipopolysaccharide (10 μg), a PAR₂-activating peptide, FLIGRL-NH₂ (30 μg), and PGE₂ (10 μg). Three hours later, groups of mice were sacrificed as described above, the trachea were cannulated, and bronchoalveolar
prodced negligible increases in PGE2 levels in A549 cell cultures (Fig. 1).

As shown in Fig. 2, A549 cell cultures exposed to 50 μM SLIGRL-NH2 for 30 min had significantly elevated levels of PGE2 (P < 0.05 compared with no SLIGRL-NH2). These acute SLIGRL-NH2-induced increases in PGE2 levels were further enhanced in A549 cell cultures that had been preexposed for 24 h to 10 μg/ml lipopolysaccharide (P = 0.001 compared with SLIGRL-NH2 alone, Fig. 2). In contrast, acute SLIGRL-NH2-induced increases in PGE2 levels were not evident in A549 cells that had been pre-exposed to dexamethasone (Fig. 2), irrespective of whether the cells had been coexposed to lipopolysaccharide.

**Results**

**Influence of Lipopolysaccharide and Dexamethasone on SLIGRL-NH2-Induced Generation of PGE2 in A549 Cell Cultures**

The PAR2-activating peptide SLIGRL-NH2 produced time- and concentration-dependent increases in PGE2 levels in the culture medium of A549 cells (Fig. 1). Increases in PGE2 levels were rapid, and within 15 min of exposure to SLIGRL-NH2, the levels of PGE2 were nearly half of the maximum levels obtained at 24 h (Fig. 1A). The concentration of SLIGRL-NH2 that produced 50% of the maximum increase in PGE2 levels (EC50 value) was approximately 35 μM (Fig. 1B). As expected, SLIGRL-NH2-induced increases in PGE2 levels were abolished by a cyclooxygenase inhibitor indomethacin, and the partially scrambled control peptide LSIGRL-NH2 produced negligible increases in PGE2 levels in A549 cell cultures (Fig. 1).

**Data Analyses.** Estimates of EC50 values were obtained by fitting each PGE2 and carbachol concentration-response curve to a four-parameter logistic function (sigmoidal curve, SigmaPlot; Systat Software, Inc., San Jose, CA). Estimates of SLIGRL-NH2 potency were determined by estimating the concentration of SLIGRL-NH2 that produced 30% relaxation of the carbachol-induced contraction. Group agonist potency data (presented in Table 1) are expressed as mean and 95% confidence limits, and different groups were compared using analysis of variance on log-transformed data. Data in the figures are presented as mean ± S.E.M. Statistically significant differences were indicated when the P value was less than 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SLIGRL-NH2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PGE2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Carbachol&lt;sup&gt;b&lt;/sup&gt;</th>
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<td></td>
<td>Control +Dex</td>
<td>Control +Dex</td>
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<td>Short-term in vitro (n = 5)</td>
<td>3.7</td>
<td>230</td>
<td>200</td>
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<td>1.7–8.1</td>
<td>2.6–5.2</td>
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<td>2.7</td>
<td>3.4</td>
<td>185</td>
<td>450</td>
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<tr>
<td>4.4</td>
<td>5.9</td>
<td>160–250</td>
<td>110–540</td>
</tr>
<tr>
<td>In vivo (n = 10–11)</td>
<td>3.2–6.0</td>
<td>100–290</td>
<td>140–240</td>
</tr>
<tr>
<td>4.7</td>
<td>4.1–8.5</td>
<td>100–330</td>
<td>150–250</td>
</tr>
<tr>
<td>In vivo + LPS (n = 10–12)</td>
<td>3.4–6.4</td>
<td>100–390</td>
<td>160–270</td>
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Dex, dexamethasone.

<sup>a</sup> Potency values for SLIGRL-NH2 represent the mean concentration that produced 30% relaxation of the carbachol-induced contraction.

<sup>b</sup> Potency values for PGE2 and carbachol represent the mean concentration that produced EC50.
vitro exposure to 1 μM dexamethasone was not associated with any significant change in carbachol potency (Fig. 4C; Table 1).

In Vivo Exposure to Dexamethasone. In vitro responsiveness to carbachol, SLIGRL-NH₂, and PGE₂ was also determined in tracheal preparations obtained from mice that had been given an i.p. injection of 3 mg/kg dexamethasone (or vehicle) 24 h before tracheal resection. As shown in Fig. 5, in vitro responsiveness to SLIGRL-NH₂, PGE₂, and carbachol determined in tracheal preparations obtained from dexamethasone-treated mice was similar to that in preparations from vehicle-treated mice (Fig. 5; Table 1). Likewise, in mice exposed to lipopolysaccharide, in vivo administration of dexamethasone did not modulate the in vitro responsiveness of tracheal preparations to SLIGRL-NH₂, PGE₂ or carbachol (Fig. 6; Table 1).

Influence of Dexamethasone on f-LIGRL-NH₂-Induced Inhibition of Lipopolysaccharide-Induced BAL Neutrophilia in BALB/c Mice. As expected, intranasal administration of lipopolysaccharide induced a marked inflammatory response in mice, characterized by the appearance of large numbers of neutrophils in BAL fluid (Fig. 7A). The numbers of neutrophils were significantly lower in BAL fluid recovered from lipopolysaccharide-exposed mice that had been treated with either the PAR₂-activating peptide f-LIGRL-NH₂ (50 ± 11% fewer neutrophils, n = 6, P < 0.001) or dexamethasone (60 ± 3% fewer neutrophils, n = 6, P < 0.001) (Fig. 7A). Of particular interest, even fewer BAL neutrophils were obtained from lipopolysaccharide-exposed mice that received both f-LIGRL-NH₂ and dexamethasone (80 ± 7% fewer neutrophils, P = 0.01 compared with lipopolysaccharide-exposed mice that received either f-LIGRL-NH₂ or dexamethasone alone) (Fig. 7A). A similar profile of response was observed in mice that received PGE₂ instead of f-LIGRL-NH₂ (Fig. 7B). That is, the numbers of BAL neutrophils recovered from lipopolysaccharide-exposed mice were reduced by 54 ± 5% with PGE₂ alone (n = 7), by 40 ± 5% with dexamethasone alone (n = 7), and by 83 ± 3% with PGE₂ plus dexamethasone (n = 8, P = 0.005, Fig. 7B).

Discussion

There is a large body of compelling data indicating that airway epithelial cell cultures exposed to glucocorticoids have suppressed PGE₂ levels (Mitchell et al., 1994; Newton et al.,
In these epithelial cell cultures, glucocorticoids inhibit PGE2 production by suppressing the release of arachidonic acid, an initial substrate in the synthesis of PGE2 (Chivers et al., 2004), and by inhibiting the expression of PGES and COX, which convert arachidonic acid into PGE2 (Mitchell et al., 1994; Newton et al., 1997; Chivers et al., 2004). In addition, glucocorticoids enhance the metabolism of PGE2 by increasing the expression of prostaglandin dehydrogenase (Tong and Tai, 2005). This raises the possibility that glucocorticoids will suppress the activity of substances, such as PAR2 activators, whose bronchoprotective actions are predominantly mediated via the production of PGE2 (Cocks et al., 1999; Lan et al., 2001; De Campo and Henry, 2005). Consistent with this postulate, we observed that PAR2-mediated, SLIGRL-NH2-induced increases in PGE2 levels in airway epithelial cell cultures were abolished by the glucocorticoid, dexamethasone. However, despite these impressive inhibitory effects in cultured cells, dexamethasone did not inhibit relaxation responses induced by SLIGRL-NH2 in intact airway smooth muscle preparations, irrespective of whether the responses were obtained after short-term in vitro exposure, long-term in vitro exposure, or ...

Fig. 3. Concentration response curves for SLIGRL-NH2 (A), PGE2 (B), and carbachol (C) in mouse isolated tracheal smooth muscle preparations after short-term exposure to 1 μM dexamethasone (○) or vehicle (●). Short-term exposure involved the administration of dexamethasone or vehicle to the tissue bath 15 min before determination of relaxant responses to SLIGRL-NH2 and PGE2 and contractile responses to carbachol. Shown are the mean ± S.E.M. responses obtained from five mice.

Fig. 4. Concentration response curves for SLIGRL-NH2 (A), PGE2 (B), and carbachol (C) in mouse isolated tracheal smooth muscle preparations after longer term exposure to 1 μM dexamethasone (○) or vehicle (●). Longer term exposure involved administering dexamethasone or vehicle to tissue culture media surrounding tracheal segments for 3 days, before determination of relaxant responses to SLIGRL-NH2 and PGE2 and contractile responses to carbachol. Shown are the mean ± S.E.M. responses obtained from five mice.
in vivo exposure to dexamethasone. Furthermore, dexamethasone significantly enhanced the inhibitory effect of another PAR2-activating peptide f-LIGRL-NH₂ on lipopolysaccharide-induced neutrophilia in mice. Thus, although dexamethasone blocked PAR2-mediated effects in cultured epithelial cells, these inhibitory effects did not seem to be translated through to more complex biological systems.

In the current study, addition of SLIGRL-NH₂ to the culture medium of unstimulated A549 cells produced time- and concentration-dependent increases in PGE₂ levels. PGE₂ was produced rapidly, with half-maximal levels of PGE₂ obtained within 15 min of exposure to SLIGRL-NH₂. Consistent with this, Kawao et al. (2005) recently demonstrated a time and concentration dependence of SLIGRL-NH₂-induced PGE₂ production in A549 cells. Furthermore, a putative activator of PAR₂, neutrophil elastase also stimulated the rapid release of PGE₂ from primary cultures of human bronchial epithelial cells (Perng et al., 2003). These rapid responses indicate that A549 cells constitutively express the signaling pathways required for PAR₂-mediated production of PGE₂. SLIGRL-NH₂-induced production of PGE₂ in A549 cells seemed to be mediated via PAR₂ because the partially scrambled peptide LSIGRL-NH₂ was inactive (current study; Asokananthan et al., 2002; Kawao et al., 2005).

Initial events in PAR₂-mediated generation of PGE₂ include the activation of mitogen-activated protein kinase and...
cytosolic phospholipase A2 and the resultant generation of arachidonic acid (Kawao et al., 2005). Of particular relevance, dexamethasone has been reported to rapidly inhibit arachidonic acid (and hence PGE2) release by inhibiting key components in the signal transduction pathway that lead to the activation of mitogen-activated protein kinase and cytosolic phospholipase A2 (Choudhury et al., 2000; Chivers et al., 2004). Indeed, in the current study, pretreatment with dexamethasone significantly suppressed the levels of PGE2 produced by A549 cells in response to short periods of PAR2 activation by SLIGRL-NH2. Additional studies are required to identify the underlying mechanisms through which dexamethasone blocks PAR2-mediated increases in PGE2 levels in cultured epithelial cells.

Airway epithelial cell cultures exposed to proinflammatory stimuli, including lipopolysaccharide, interleukin-1β, and tumor necrosis factor-α, release large amounts of PGE2 into the surrounding media (Mitchell et al., 1994; Newton et al., 1998, 2002; Rodgers et al., 2002; Petrovic et al., 2006). In the current study, SLIGRL-NH2-induced increases in PGE2 levels were augmented in A549 cells previously exposed to lipopolysaccharide. These findings are consistent with the synergistic effects produced by the cytokine interleukin-1β and bradykinin on PGE2 levels in epithelial cell cultures (Saunders et al., 1999; Newton et al., 2002). Together, these findings support the postulate that concomitant activation of extracellular signal-regulated kinase (in the current study via PAR2; Kawao et al., 2005) and induction of COX-2 by lipopolysaccharide stimulates maximum PGE2 synthesis in human airway epithelial cells (Petrovic et al., 2006). These synergistic effects of SLIGRL-NH2 and lipopolysaccharide on PGE2 production were not observed in cells cotreated with dexamethasone, probably due to the well documented inhibitory actions of glucocorticoids on cytokine-induced expression of important components of the PGE2 synthetic pathway, including COX-2 (Mitchell et al., 1994; Aksoy et al., 1999; Chivers et al., 2006).

PAR2-activating peptides such as SLIGRL-NH2 also stimulate the release of PGE2 in intact airway preparations such as the mouse trachea (Lan et al., 2001; Henry et al., 2005). PAR2-mediated release of PGE2 from mouse trachea induces a marked smooth muscle relaxation response (Cocks et al., 1999; Lan et al., 2000, 2001; Kawabata et al., 2004a). In the current study, acute dexamethasone exposure did not inhibit SLIGRL-NH2-induced relaxation responses in mouse isolated tracheal preparations. Thus, although dexamethasone can, within minutes, inhibit key components in the signal transduction pathway leading to PGE2 synthesis in cell culture systems (Choudhury et al., 2000; Croxtall et al., 2000, 2002; Chivers et al., 2004), this effect was not evident in the intact airway preparations. In further studies, a tissue culture system was used to determine whether longer term in vitro exposure to dexamethasone altered the responsiveness of intact airway preparations to the PGE2-dependent relaxant actions of PAR2-activating peptides (Bachar et al., 2005). In these studies, mouse isolated tracheal preparations exposed for 3 days to dexamethasone were no more or less sensitive to SLIGRL-NH2 or PGE2 than untreated preparations. Likewise, in vivo administration of dexamethasone to mice did not alter the in vitro sensitivity of tracheal preparations to either SLIGRL-NH2 or PGE2. Thus, neither short-term nor longer term in vitro exposure to dexamethasone, nor in vivo exposure to dexamethasone, had any significant inhibitory effect on the bronchodilatory PAR2-PGE2-EP2 receptor axis in mouse isolated trachea.

In the current study, dexamethasone blocked PAR2-mediated production of PGE2 in A549 cells but did not exert any inhibitory effect on PAR2-mediated relaxations in mouse isolated tracheal preparations. One possible explanation for these differential effects of dexamethasone is that PGE2 synthesis was mediated by dexamethasone-sensitive COX-2 in A549 cells, whereas the relaxations of the murine tracheal smooth muscle were mediated by dexamethasone-insensitive COX-1. However, several comprehensive studies have demonstrated that PAR2-mediated production of PGE2 by A549 cells (Kawao et al., 2005) and PAR2-mediated relaxation in mouse isolated tracheal segments (Kawabata et al., 2004) are mediated by both COX-1 and COX-2. Thus, it is unlikely that the current findings can be explained on the basis of dexamethasone.
methasone selectively suppressing the expression/function of COX-2 because COX-2 is involved in PAR2-mediated relaxation of mouse isolated tracheal preparations (Lan et al., 2001; Kawabata et al., 2004), and COX-1 is involved in PAR2-mediated generation of PGE2 in A549 cells (Kawao et al., 2005).

A major focus of the tension recording studies described above was to determine the influence of dexamethasone on relaxation responses induced by a PAR2-activating peptide SLIGRL-NH2. Concurrent experiments examined the effect of dexamethasone on relaxations induced by exogenous PGE2. These data indicate that neither in vivo nor in vitro exposure of trachea to dexamethasone inhibited the PGE2 EP receptor-relaxation pathway. Furthermore, we determined the influence of dexamethasone on carbachol concentration-response curves. The consistent finding that dexamethasone had no significant modulatory influence on carbachol-induced contractions was important because both the potency of, and maximum response to, bronchorelaxant agents such as SLIGRL-NH2 and PGE2 will be sensitive to changes in the level of spasmodogen-induced tone.

In vivo administration of SLIGRL-NH2 inhibits lipopolysaccharide-inhibited neutrophil influx into the lungs of BALB/c mice (Moffatt et al., 2002). In the current study, a similar inhibitory response was produced by f-LIGRL-NH2, a structurally related PAR2-activating peptide. f-LIGRL-NH2 was used in these latter experiments because it is a more potent and metabolically stable PAR2-activating peptide than SLIGRL-NH2 (Kawabata et al., 2004b), and thus better suited to in vivo studies. Additional studies were conducted to determine whether f-LIGRL-NH2-induced inhibition of neutrophil infiltration was modulated by cotreatment with the glucocorticoid dexamethasone. In these studies, pretreatment of lipopolysaccharide-exposed mice with dexamethasone alone was associated with reduced BAL neutrophil numbers, as previously demonstrated by others (Le-fort et al., 2001; Birrell et al., 2004; Kang et al., 2006; Moschos et al., 2007). Of particular interest, the inhibitory effect of f-LIGRL-NH2 on neutrophil infiltration in lipopolysaccharide-exposed mice was significantly greater in mice that were cotreated with dexamethasone. Consistent with a previous report (Goncalves de Moraes et al., 1996), intranasal PGE2 significantly reduced BAL neutrophil numbers in lipopolysaccharide-exposed mice. Moreover, this effect was further enhanced by dexamethasone cotreatment. The underlying mechanism for this interaction among lipopolysaccharide, glucocorticoids, and PGE2 (exogenous or PAR2-mediated) is not clear, although recent evidence implicates the transcription factor NF-κB as a pivotal player in this process. For example, NF-κB plays an important role in lipopolysaccharide-induced inflammatory responses (for review, see Doyle and O’Neill, 2006), and the level of activation of NF-κB in lipopolysaccharide-exposed cells has been reported to be suppressed by both glucocorticoids (Eddleston et al., 2007; Newton and Holden, 2007) and PGE2 (Gomez et al., 2005). Regardless of whether this represents an additive or synergistic effect between the PAR2-activating peptide/PGE2 and the glucocorticoid, it was clear that their combined effects were altogether greater than their individual activities, and there was no indication that the anti-inflammatory actions of f-LIGRL-NH2 or PGE2 were at all suppressed by dexamethasone. On a side note, although PAR2-activating peptides have been reported to increase the numbers of neutrophils in control mice (Su et al., 2005), there was no indication in the current study that intranasal f-LIGRL-NH2 produced any significant increase in BAL neutrophil numbers above that produced by vehicle in mice that were not exposed to lipopolysaccharide.

In summary, the current study clearly demonstrates that dexamethasone does not inhibit PAR2-dependent, PGE2-mediated relaxation responses in murine airways and, to the contrary, may augment anti-inflammatory responses to PAR2-activating peptides in the lipopolysaccharide-exposed lung. Thus, agents that elevate endogenous levels of PGE2 and/or stimulate downstream EP receptors are likely to retain their bronchoprotective activity in the presence of coadministered glucocorticoid pharmacotherapy.

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increases IL-8 generation in airway epithelial cells via COX-2-derived prostanoioids.
activated receptor-2 activation induces acute lung inflammation by neuropeptide-

Address correspondence to: Dr. Peter J. Henry, School of Medicine and Pharmacology, University of Western Australia, Stirling Highway, Nedlands, Australia 6009. E-mail: Peter.Henry@uwa.edu.au