Inhibitors of Prostaglandin Transport and Metabolism Augment Protease-Activated Receptor-2-Mediated Increases in Prostaglandin E2 Levels and Smooth Muscle Relaxation in Mouse Isolated Trachea

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ABSTRACT

Stimulants of protease-activated receptor-2 (PAR2), such as Ser-Leu-Ile-Gly-Arg-Leu-NH2 (SLIGRL), cause airway smooth muscle relaxation via the release of the bronchodilatory prostanoid prostaglandin E2 (PGE2). The principal aim of the current study was to determine whether compounds that inhibit PGE2 reuptake by the prostaglandin transporter [bromocresol green and U46619 (9,11-dideoxy-9\(\frac{1}{2}\)/H9251,11\(\frac{1}{2}\)/H9251-methanoepoxy PGF2\(\frac{1}{2}\)/H9251)] and PGE2 metabolism by 15-hydroxyprostaglandin dehydrogenase (thiazolidenedione compounds rosiglitazone and ciglitazone) significantly enhanced the capacity of SLIGRL to elevate PGE2 levels and produce relaxation in isolated segments of upper and lower mouse trachea. SLIGRL produced concentration-dependent increases in PGE2 levels and smooth muscle relaxation, although both effects were significantly greater in lower tracheal segments than in upper tracheal segments. SLIGRL-induced increases in PGE2 levels were significantly enhanced in the presence of ciglitazone and rosiglitazone, and these effects were not inhibited by GW9662 (2-chloro-5-nitrobenzanilide), a peroxisome proliferator-activated receptor-\(\gamma\) antagonist. SLIGRL-induced relaxation responses were also significantly enhanced by ciglitazone and rosiglitazone, whereas responses to isoprorenaline, a PGE2-independent smooth muscle relaxant, were unaltered. Ciglitazone and rosiglitazone alone produced concentration-dependent increases in PGE2 levels and smooth muscle relaxation, and these responses were inhibited by indomethacin, a cyclooxygenase inhibitor. Bromocresol green, an inhibitor of prostaglandin transport, significantly enhanced SLIGRL-induced increases in PGE2 levels and relaxation. Immunohistochemical staining for 15-hydroxyprostaglandin dehydrogenase was relatively intense over airway smooth muscle, as was staining for the prostaglandin transporter over both airway smooth muscle and epithelium. In summary, inhibitors of PGE2 reuptake and metabolism significantly potentiate PAR2-mediated increases in PGE2 levels and smooth muscle relaxation in murine-isolated airways.

Protease-activated receptors (PARs) are widely expressed within the airways, and activation of particular PAR subtypes significantly modulates cellular activity. Moreover, there is accumulating evidence that PARs play a role in the pathogenesis of airway diseases, although there is considerable uncertainty as to whether it is activation or blockade of PARs that offers greater potential benefit (for recent reviews, see Lan et al., 2002; Moffatt et al., 2004; Ossovskaya and Bunnett, 2004; Kawabata and Kawao, 2005).

Small peptide activators of PAR2, such as SLIGRL, have recently been shown to exhibit anti-inflammatory effects in a variety of murine models of airway disease, including bacterial lipopolysaccharide-induced neutrophilia (Moffatt et al., 2002) and ovalbumin-induced eosinophilia and hyperresponsiveness (De Campo and Henry, 2005). In addition, PAR2 activators inhibit methacholine-induced bronchoconstriction in allergic and viral models of airways disease (Lan et al., 2004; De Campo and Henry, 2005), which is consistent with the relaxation responses produced in isolated airway smooth muscle preparations (Cocks et al., 1999; Lan et al., 2000; Kawabata et al., 2004). Many of these in vivo and in vitro bronchoprotective effects of PAR2 activators are inhibited by indomethacin, indicating the involvement of cyclooxygenase.
products (Cocks et al., 1999; Lan et al., 2000, 2004; Kawabata et al., 2004; De Campo and Henry, 2005). In this regard, prostaglandin (PG) E₂ is a prime candidate because it is generated by the airways in response to PAR₂ activation (Lan et al., 2001) and mimics many of the effects of SLIGRL (De Campo and Henry, 2005).

PAR₂ activators stimulate the release of PGE₂ from epithelial cells (Asokananthan et al., 2002). Following its release, PGE₂ activates various cell-surface E prostanoan (EP) receptors (EP₁–EP₄) to produce a surprisingly wide range of effects, including many that limit bronchoconstriction and inflammation (for review, see Vancheri et al., 2004). As expected for a hormone that exerts powerful local effects, the biological activity of PGE₂ is strictly controlled. The compartmentation of PGE₂ signaling occurs through a two-step process involving intracellular uptake, followed by oxidation to less active metabolites (Nomura et al., 2004). PGE₂, like other prostaglandins, predominates as a charged anion, and thus a carrier-mediated transport system is required for it to cross membranes. The prostaglandin transporter belongs to a superfamily of 12-transmembrane organic anion transporting polypeptides and catalyzes the rapid, specific, and high-affinity uptake of PGE₂, PGD₂, and PGF₂α. The prostaglandin transporter can be inhibited by bromocresol green (Banan et al., 2003) and a synthetic thromboximetric U46619 (Itoh et al., 1996; Pucci et al., 1999).

Once PGE₂ has been transported into the cell, it is rapidly oxidized to 15-ketoPGE₂, which possesses greatly reduced biological activities. The key metabolic enzyme controlling PGE₂ oxidation is a cytosolic enzyme, 15-hydroxyprostaglandin dehydrogenase. Interestingly, among the most potent inhibitors of 15-hydroxyprostaglandin dehydrogenase are ciglitazone and rosiglitazone, two thiazolidinedione compounds that are widely recognized as activators of peroxisome proliferator-activated receptor-γ (PPAR-γ). Indeed, the concentrations of ciglitazone and rosiglitazone needed to inhibit 15-hydroxyprostaglandin dehydrogenase are similar to the concentration required to activate PPAR-γ (Cho and Tai, 2002a). Several recent studies have demonstrated that these thiazolidinedione compounds inhibit pulmonary eosinophilia and hyperresponsiveness in murine models of allergic airways inflammation (Mueller et al., 2003; Trifilieff et al., 2003; Weerly et al., 2003; Hammad et al., 2004; Honda et al., 2004). The relative contribution of PPAR-γ activation and 15-hydroxyprostaglandin dehydrogenase inhibition to these effects is not clear.

In summary, activators of airway PAR₂ stimulate the release of PGE₂, which exerts a range of powerful bronchoprotective activities. However, PGE₂ signaling is rapidly terminated by a series of specific reuptake and metabolic processes. In the current study, we investigated whether compounds that inhibit PGE₂ reuptake by a prostaglandin transporter and PGE₂ metabolism by 15-hydroxyprostaglandin dehydrogenase significantly enhance the capacity of PAR₂ activators such as SLIGRL to elevate PGE₂ levels and produce airway smooth muscle relaxation.

**Materials and Methods**

**Mouse Isolated Tracheal Preparations.** Female BALB/c mice at 6 to 8 weeks of age were killed by an overdose of pentobarbital sodium (250 mg kg⁻¹) (Rhone Merieux Australia Pty Ltd, Pinkenba, Australia), and the trachea was removed by careful surgical resection. The trachea was bisected, and the resultant upper and lower segments were suspended under a resting tension of 300 mg in separate organ baths containing 1 ml of Krebs bicarbonate solution maintained at 37°C and bubbled with 5% CO₂ in O₂. The composition of the Krebs bicarbonate solution was as follows: 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 Na-glucose. Changes in tension were recorded via an FT03 isometric force transducer (Grass Instruments, Quincy, MA) connected to a PowerLab data acquisition and analysis system (ADInstruments, Castle Hill, Australia). Following a 40-min equilibration period, tracheal segments were challenged with cumulative concentrations of 0.2 and 10 μM carbachol. Once the contraction induced by 10 μM carbachol had reached a plateau (maximum carbachol-induced contraction, Cmax), preparations were washed repeatedly and rested for 15 min. Following the 15-min rest period, tracheal segments were incubated with fresh Krebs bicarbonate solution containing one of the following compounds: 10 μM ciglitazone, 10 μM rosiglitazone, 30 μM bromocresol green, 10 μM GW9662, 2.5 μM indomethacin, or vehicle (0.8% saline or 0.1% DMSO in Krebs bicarbonate solution). Each compound was left in contact with the tracheal segment for 15 min, and then the bathing solution replaced with fresh Krebs bicarbonate solution containing the same compound. Preparations were then used for PGE₂ or relaxation studies, as described below.

**PGE₂ Studies.** Segments were prepared as described above and then contracted using 0.5 μM carbachol (or 1 μM U46619 in selected studies). Five minutes later, a 10-μl volume of saline (SLIGRL vehicle) was added to the bath (15% by volume). Ten minutes later, the entire bathing fluid was collected and frozen (−20°C) until PGE₂ levels were assayed (basal PGE₂ levels). Tracheal segments were washed and rested in fresh (drug-free) Krebs bicarbonate solution for 15 min before the process was repeated three times using the same test compound, but instead of exposing the segments to saline, they were exposed sequentially to either 20 μM SLIGRL, 80 μM SLIGRL, or 80 μM LSIGRL (a partially scrambled control peptide). Thus, four samples were collected from each tracheal segment (saline, 20 μM SLIGRL, 80 μM SLIGRL, and 80 μM LSIGRL in the presence of one of the compounds listed above. The level of PGE₂ in each of the samples was determined using a commercial PGE₂ EIA kit (Cayman Chemical, Ann Arbor, MI) in accordance with the manufacturer’s instructions. PGE₂ levels were expressed as picograms of PGE₂ collected over 15 min per tracheal segment.

**Relaxation Studies.** Segments were prepared as described above and contracted with 0.5 μM carbachol (or 1 μM U46619 in selected experiments). When the contraction had reached a plateau, segments were exposed to 10 μM SLIGRL. Once the relaxation response had peaked, tracheal segments were washed and rested for 15 min. This cycle was repeated four times, exposing precontracted preparations sequentially to 20, 40, and 80 μM SLIGRL and to 80 μM LSIGRL. Contractile responses to carbachol and U46619 were expressed as a percentage of Cmax, and relaxation responses were expressed as a percentage of reversal of the level of precontraction. In additional experiments, concentration-response curves to isoprenaline (10−1000 nM), PGE₂ (3−1000 nM), 8-Br-cAMP (3−1000 μM), rosiglitazone (5−20 μM), and ciglitazone (5−20 μM) were obtained in carbachol-contracted mouse isolated tracheal segments.

**Immunohistochemical Studies.** Following pulmonary perfusion with 0.2% picric acid/2% paraformaldehyde in phosphate-buffered saline, pH 7.4, lungs and tracheas were postfixed in the above fixative for 4 h at 4°C. Tissue was subsequently washed, dehydrated, and embedded in paraplast. The expression of prostaglandin transporter and 15-hydroxyprostaglandin dehydrogenase was determined by immunoperoxidase using the DAKO EnVision System kit (DakoCytomation California Inc., Carpinteria, CA), polyclonal rabbit anti-human prostaglandin transporter, polyclonal rabbit anti-15-hydroxyprostaglandin dehydrogenase, and blocking peptides specific for both antibodies (Cayman Chemical). Briefly, 5-μm sections were...
permeabilized with 1% Triton X-100 in 50 mM Tris buffer plus 0.5 M NaCl, pH 7.4, for 20 min. Endogenous peroxidase was then quenched with 3% H₂O₂ in 50 mM Tris buffer for 10 min. One-hundred percent normal goat serum block (Vector Laboratories, Burlingame, CA) for 15 min was followed by overnight incubation at 4°C with the primary antibody or antibody-blocking peptide solution at the following concentrations: 30 μg/ml antiprostaglandin transporter, 50 μg/ml antiprostaglandin transporter blocking peptide, 10 μg/ml anti-15-hydroxyprostaglandin dehydrogenase, and 15 μg/ml anti-15-hydroxyprostaglandin dehydrogenase-blocking peptide. Incubation with the polymer-labeled secondary antibody and substrate chromogen complex followed the recommendations of DakoCytomation California Inc. Finally, the tissue was briefly counterstained with Mayer’s hematoxylin. Sections were examined by brightfield microscopy (Olympus BX50), and images were captured with an Olympus DP11 digital camera system (Olympus, Tokyo, Japan).

Data Analysis and Statistics. Grouped data are expressed as mean ± S.E.M. Concentration-response curves were compared by two-way repeated measures ANOVA using SigmaStat (SPSS Inc., Chicago, IL); p values of less than 0.05 were considered statistically significant.

Drugs. Carbamylcholine chloride (carbachol), isoprenaline, ciglitazone, bromocresol green, GW9662, and indomethacin were obtained from Sigma-Aldrich (St. Louis, MO), and rosiglitazone, ciglitazone, bromocresol green, GW9662, and indomethacin were obtained from Cayman Chemical. SLIGRL-NH₂ and LSIGRL-NH₂ were purchased from Proteomics International (Perth, Australia).

Results

SLIGRL-Induced Increases in PGE₂ Levels and Tracheal Smooth Muscle Relaxation. Following a 15-min incubation period, significant levels of PGE₂ were detected in the medium surrounding unstimulated segments of upper trachea (32 ± 5 pg/segment, n = 6). As shown in Fig. 1A, these basal PGE₂ levels were not significantly different from those observed in segments of lower trachea (38 ± 6 pg/segment, n = 7; one-way ANOVA).

SLIGRL induced concentration-dependent increases in PGE₂ levels, although significantly higher levels of PGE₂ were recovered from the medium surrounding lower tracheal segments (80 μM SLIGRL, 147 ± 15 pg/segment, n = 12) than upper tracheal segments (80 μM SLIGRL, 72 ± 12 pg/segment, n = 12; two-way ANOVA; Fig. 1A). Consistent with this finding, the concentration-dependent relaxation responses induced by SLIGRL in tracheal segments precontracted with 1 μM carbachol were significantly greater in lower tracheal segments than in upper tracheal segments (Fig. 1B). Relaxation responses induced by PGE₂ (Fig. 1C) and 8-Br-cAMP (Fig. 1D) were similar in lower and upper tracheal segments. The level of preconstriction induced by 0.5 μM carbachol was similar in lower (72.7 ± 1.6%, n = 88) and upper (72.6 ± 1.2%, n = 93) tracheal segments.

The partially scrambled peptide LSIGRL (80 μM) did not induce any relaxant (or contractile) response in either upper or lower tracheal segments. Incubation with LSIGRL was associated with the production of PGE₂ levels (80 μM LSIGRL; 48 ± 5 pg/lower segment, n = 4; 55 ± 9 pg/upper segment, n = 6) that were not significantly different from basal PGE₂ levels but were less than those induced by 80 μM SLIGRL (one-way ANOVA).

Potentiation of SLIGRL-Induced Responses by Rosiglitazone and Ciglitazone. In the presence of 10 μM rosiglitazone, SLIGRL-induced release of PGE₂ was augmented in both upper and lower segments of the trachea (Fig. 2, A and B; two-way repeated measures ANOVA; p < 0.05). Similarly, rosiglitazone (1 and 10 μM) potentiated SLIGRL-induced relaxation responses in both tracheal regions (Fig. 2, C and D; p < 0.05). A related thiazolidinedione, ciglitazone (10 μM), also potentiated SLIGRL-induced increases in both PGE₂ levels (Fig. 2, A and B; p < 0.05) and relaxation responses (Fig. 2, E and F; p < 0.05).

Because rosiglitazone and ciglitazone have well recognized activity as stimulants of PPAR-γ, we examined the effects of the PPAR-γ antagonist GW9662 on the effects produced by these thiazolidinediones. GW9662 (10 μM) did not inhibit the effects produced by rosiglitazone or ciglitazone on SLIGRL-induced elevations in PGE₂ levels (Fig. 3, A–D). The effects of GW9662 on rosiglitazone- or ciglitazone-induced potentiation of SLIGRL-induced relaxation were not examined because GW9662 caused a concentration-dependent inhibition of SLIGRL-induced relaxation in both upper (Fig. 3E; p < 0.05) and lower (Fig. 3F; p < 0.05) tracheal segments. Although rosiglitazone potentiated SLIGRL-induced relaxation responses, it did not modulate responses induced by isoprenaline (β-adrenoceptor agonist) in either upper (Fig. 4A) or lower (Fig. 4B) segments of mouse isolated trachea.

Rosiglitazone caused a concentration-dependent increase in PGE₂ levels (Fig. 5A) and relaxation (Fig. 5B) in upper and lower tracheal segments. These effects were blocked by 2.5 μM indomethacin, a nonselective inhibitor of cyclooxygenase. As expected from our previous studies (Lan et al., 2001), indomethacin also blocked SLIGRL-induced increases in PGE₂ levels and relaxation responses. Carbachol-induced contractions were significantly larger in tracheal prepara-
tions incubated with indomethacin (80.9 ± 3.6, n = 12) than vehicle (72.6 ± 1.0, n = 181; one-way ANOVA; p < 0.05).

Potentiation of SLIGRL-Induced Responses by Bromocresol Green. An inhibitor of prostaglandin transporter, bromocresol green (30 μM), significantly potentiated SLIGRL-induced increases in PGE2 levels (Fig. 6, A and B; two-way repeated measures ANOVA; p < 0.05) and relaxation responses (Fig. 6, C and D; p < 0.05) in carbachol-contracted tracheal segments. We also examined the effects of SLIGRL in tracheal segments that were precontracted with U46619, a thromboxane A4 analog that has dual activities as a smooth muscle spasmogen and inhibitor of the prostaglandin transporter. Interestingly, SLIGRL-induced relaxation responses were significantly greater in lower tracheal preparations precontracted with 1 μM U46619 than 0.5 μM carbachol (Fig. 6F; p < 0.05), although PGE2 levels were similar (Fig. 6B).

Immunohistochemical Localization of 15-Hydroxyprostaglandin Dehydrogenase and Transporter. As Fig. 7 clearly demonstrates, high levels of immunostaining for the prostaglandin transporter were associated with both the tracheal epithelial layer and the smooth muscle band (Fig. 7A). In contrast, immunostaining for 15-hydroxyprostaglandin dehydrogenase was localized to the smooth muscle band, with no evidence of epithelial staining (Fig. 7C). Control sections incubated with specific blocking peptides were unstained, confirming the specificity of the reaction (Fig. 7, B and D).

Discussion

The current study clearly demonstrates that activators of PAR2 stimulate PGE2 release from murine isolated trachea and that this effect is enhanced significantly by inhibition of
PGE₂ reuptake or metabolism. Moreover, these elevated PGE₂ levels were associated with enhanced smooth muscle relaxation responses. These novel findings support the postulate that augmentation of endogenous PGE₂ levels through the combined effects of enhanced production (via PAR₂) and reduced degradation may be a novel means of exploiting the well-established bronchoprotective effects of PGE₂.

In the current study, the assay of the physiological solution surrounding mouse isolated tracheal segments revealed significant levels of the prostanoid PGE₂. Pretreatment of these preparations with a cyclooxygenase inhibitor, indomethacin, reduced PGE₂ levels to below the detection limit. These findings indicate that unstimulated tracheal segments synthesize and release PGE₂. The physiological role of basal PGE₂ release is not known. However, carbachol-induced contractions were significantly larger in indomethacin-treated preparations, suggesting that basal PGE₂ release is sufficient to inhibit spasmogen-induced constriction. Whether basal levels of PGE₂ are also sufficient to blunt inflammatory and immune responses within the airways is not known.

Incubation of mouse isolated tracheal preparations in physiological solution containing either of the thiazolidinedione compounds rosiglitazone or ciglitazone was associated with the detection of elevated levels of PGE₂. This is consistent with the recent finding in cell-free systems that rosiglitazone and ciglitazone inhibit 15-hydroxyprostaglandin dehydrogenase (Cho and Tai, 2002a). In functional studies, ciglitazone and rosiglitazone produced concentration-dependent relaxation responses in carbachol-contracted preparations, which were inhibited by indomethacin. Together, these findings indicate that thiazolidinediones elevate the levels of PGE₂ by inhibiting 15-hydroxyprostaglandin dehydrogenase and thereby functionally antagonizing carbachol-induced contractions.

Ciglitazone and rosiglitazone have been used widely—clinically and experimentally—as activators of PPAR-γ, a transcription factor thought to play a prominent role in lipogenesis and adipocyte differentiation (Ferre, 2004; Rangwala and Lazar, 2004). Although the concentrations of these thiazolidinediones that activate PPAR-γ and inhibit 15-hydroxyprostaglandin dehydrogenase are comparable (Cho and Tai, 2002b), it is unlikely that PPAR-γ activation played a significant role in ciglitazone- or rosiglitazone-induced increases in PGE₂ levels. First, the effects of these thiazolidinediones on PGE₂ levels were not inhibited by GW9662, an irreversible PPAR-γ antagonist (Leesnitzer et al., 2002). Second, the effects of ciglitazone and rosiglitazone were observed far more quickly (within 15 min) than would be expected for an effect requiring gene transcription.

Another compound that increased PGE₂ levels in unstimulated mouse isolated tracheal preparations was bromocresol green, an inhibitor of the prostaglandin transporter (Banu et al., 2003). These findings support a currently held view that PGE₂ removal is a two-step process—PGE₂ uptake via a prostaglandin transporter followed by intracellular degradation by 15-hydroxyprostaglandin dehydrogenase. Immunohistochemical studies revealed high levels of the prostaglandin transporter over tracheal epithelium and smooth muscle and 15-hydroxyprostaglandin dehydrogenase over smooth muscle. One possible explanation for these findings is that PGE₂ is synthesized and released from the tracheal epithelium (via a prostaglandin transporter) and is then taken up...
into smooth muscle by the transporter and metabolized intracellularly by 15-hydroxyprostaglandin dehydrogenase. In between its release and uptake, PGE2 can exert smooth muscle relaxant effects on smooth muscle via stimulation of EP2 receptors (Lan et al., 2001).

As indicated above, compounds that inhibit the uptake and metabolism of PGE2 cause elevated basal levels of this prostanoid. Activators of PAR2—such as SLIGRL—also elevated PGE2 levels within the airways (Lan et al., 2001). The precise mechanism(s) through which PAR2 activators elevate PGE2 levels is not clear, but it likely involves enhanced synthesis of PGE2 rather than inhibition of PGE2 uptake or metabolism. Consistent with our previous findings (Lan et al., 2000, 2001), SLIGRL caused a concentration-dependent increase in PGE2 levels and relaxation, both of which were inhibited by indomethacin. The inactivity of the partially scrambled peptide LSIGRL provides supportive evidence for the effects of SLIGRL having been mediated by PAR2 (Hollenberg, 2005).

A particularly interesting and important finding was that both the PGE2-elevating and smooth muscle-relaxing effects of SLIGRL were significantly augmented by rosiglitazone and cigitazone, most probably via a mechanism involving decreased metabolism of PGE2. This raises the intriguing possibility that agents that inhibit PGE2 metabolism will act synergistically with compounds that stimulate PGE2 release to produce powerful bronchoprotective effects within the lung. Recent studies have demonstrated that thiazolidinedione compounds have anti-inflammatory actions in murine models of allergic airways inflammation (Mueller et al., 2003; Trifilieff et al., 2003; Woerly et al., 2003; Hammad et al., 2004; Honda et al., 2004). In the study by Woerly et al. (2003), these effects were blunted by GW9662, suggesting the involvement of PPAR-γ. In several other studies, PPAR-γ antagonists were not tested, and thus the potential involvement of PPAR-γ was not confirmed. Interestingly, EP2 receptor expression was reduced in cigitazone-treated mice (Mueller et al., 2003). A possible explanation for the downregulation of the EP2 receptor is that it is an autoregulatory response to elevated levels of its natural ligand, PGE2, induced by ciglitazone. Additional in vivo studies investigating the potential synergistic interaction between agents such as rosiglitazone and PAR2 activators in allergic airways are clearly warranted.

PAR2-mediated increases in PGE2 levels and relaxation were augmented by bromocresol green, an inhibitor of the prostaglandin transporter. These findings indicate that the prostaglandin transporter plays a significant role in terminating PGE2 signaling. The actions of another inhibitor of the prostaglandin transporter, the synthetic thromboxane U46619 (Cho and Tai, 2002b), were also investigated with equivocal results. As predicted, SLIGRL-induced relaxations were greater in U64419-contracted preparations than in preparations precontracted to similar levels with carbachol. This is consistent with the hypothesis that, in the presence of U46619, the prostaglandin transporter would be inhibited and the levels of PGE2 produced in response to SLIGRL would be enhanced, resulting in a larger relaxation response. However, for reasons that are not clear, SLIGRL-induced PGE2 levels were not higher in U46619-contracted...
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In the current study, SLIGRL consistently induced significantly larger relaxation responses in segments of lower trachea than upper trachea. Regional differences were also observed in the levels of PGE₂ produced in response to SLIGRL—again, larger responses were observed in lower tracheal segments. However, no such regional differences were observed in relaxation responses induced by PGE₂ or 8-BrcAMP. Together, these findings indicate that the larger relaxation responses observed in lower tracheal segments were due to the release of greater amounts of PGE₂ rather than to an additional mechanism involving an elevated sensitivity to downstream mediators of the SLIGRL-induced response such as PGE₂ or cAMP.

GW9662 has been widely used as a selective and irreversible antagonist of PPAR-γ and, likewise, was used in the current study to investigate the involvement of PPAR-γ in the responses induced by rosiglitazone and ciglitazone. However, GW9662 caused an unexpected, concentration-dependent inhibition of SLIGRL-induced relaxation responses in both upper and lower tracheal segments. Although PPAR-γ ligands have been reported to inhibit the Rho/Rho kinase pathway involved in vascular smooth muscle contraction (Wakino et al., 2004), the mechanism(s) through which GW9662 inhibited PAR₂-mediated relaxation responses is not known. It is possible that GW9662 is acting through PPAR-γ-independent pathways (Seargent et al., 2004), although it is unlikely to be due to the inhibition of enzymes involved in PGE₂ synthesis since GW9662 did not block basal or SLIGRL-induced increases in PGE₂ (data not shown).

In summary, the current study has established that compounds that inhibit the reuptake or metabolism of PGE₂ significantly augment PAR₂-mediated relaxation responses in murine-isolated tracheal preparations. Whether these compounds—most notably the thiazolidinediones ciglitazone and rosiglitazone—augment the recently reported inhibitory effects of PAR₂ ligands in murine models of allergic inflammation remains an important topic for future research.

References


