Prostanoids Secreted by Alveolar Macrophages Enhance Ionic Currents in Swine Tracheal Submucosal Gland Cells

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Received April 27, 2005; accepted July 26, 2005

ABSTRACT

We examined the effect of substances released by swine alveolar macrophages (AMs) on ionic currents in airway submucosal gland cells (SGCs). AMs obtained by lavage were activated by 24-h zymosan exposure (0.1 mg/ml). Supernatant was collected and used to stimulate short-circuit current changes (ΔISC) in SGC monolayers in Ussing chambers. Dexamethasone (1 μM) or indomethacin (5 μM) during zymosan exposure of AMs reduced or abolished the supernatant-induced ΔISC. Zymosan exposure induced a 5-fold increase in cyclooxygenase (COX)-2 but not COX-1 protein levels in AMs. Prostaglandin E2 (PGE2) concentration in the supernatant from zymosan-activated AMs was 550 ± 10 nM (n = 3) compared with 28 ± 3 nM for unstimulated AMs (n = 3). PGE2, applied serosupran, induced ΔISC with an EC50 of 1.8 ± 0.3 μM (n = 3) when applied apically. Four types of endoprostanoid receptors (EP1-4) were detected in SGCs using Western blot. PGE2-induced ΔISC were inhibited by AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) but not by SC19220 (8-chloro-dibenzo[b,f][1,4]oxazepine-10(11H)-carboxylic acid, 2-acetylhydrazide), suggesting that endoprostanoid (EP)2 but not EP1 receptors were activated by PGE2. Pretreatment of SGCs with supernatant from zymosan-activated AMs, PGE2, or forskolin enhanced the sensitivity to acetylcholine (ACh)-induced ΔISC. PGE2-induced ΔISC were blocked by charybdotoxin (ChTX), chromanol 293B, or glibenclamide. ACh-induced ΔISC were only blocked by ChTX or glibenclamide. None of these blockers altered PGE2 pretreatment-enhanced sensitization of ACh-induced ΔISC. These results demonstrate that prostanoids released from activated AMs directly increase cystic fibrosis transmembrane conductance regulator and K+ channel activity. ACh-induced ΔISC are also enhanced due to enhanced activation of Ca2+-activated K+ channels (KCa).

Airway surface liquid and macrophages are part of the innate defense system in the airway and are important in the clearance of inhaled environmental particulates and pathogens. Air surface liquid, which forms a barrier to inhaled particles, is comprised of bacteriostatic fluid and mucus secreted mainly by SGCs (Knowles and Boucher, 2002; Verkman et al., 2003). Alveolar macrophages (AMs) phagocytize particulates and pathogens, release substances that regulate the function of the immune system, and initiate adaptive immune responses in the lung (Sibille and Reynolds, 1990; Lohmann-Matthes et al., 1994).

AMs activated by particulates release cytokines, such as interleukin-1, -6, the chemokine macrophage inflammatory protein-1α, hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor, and reactive oxygen species (Becker et al., 1996; Dorger and Krombach, 2000; Suwa et al., 2002). Activation of AMs in the lung by particulates has been shown to induce systemic effects via the circulation by increasing leukocytosis in the bone marrow (van Eeden and Hogg, 2002). Media taken from cocultures of macrophages and epithelial cells exposed to particulates and instilled into rabbit lung stimulate bone marrow (Fujii et al., 2002). AMs can also alter the function of respiratory system. For example, AM-released mediators enhance the responsiveness of rat lungs to muscarinic stimulation (Padrid et al., 1993) and particulate matter exposure of naive mice increases airway reactivity (Walters et al., 2001). Little is known however about the effects of AM-derived products in regulating epithelial function and airway fluid and mucus secretion.

We hypothesize that activated AMs release a substance or substances, which directly induce ΔISC across SGC monolayer.

ABBREVIATIONS: SGC, submucosal gland cell; AM, alveolar macrophage; ΔISC, short circuit current; ACh, acetylcholine; PG, prostaglandin; COX, cyclooxygenase; CFTR, cystic fibrosis transmembrane conductance regulator; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; DPC, diphenylamine-2-carboxylate; DIDS, disodium 4,4'-dilithiocyanostilbene-2,2'-disulfonate; TBS, Tris-buffered saline; TBST, TBS/Tween 20; EP, endoprostanoid; AH6809, 6-isoproxy-9-oxoxanthene-2-carboxylic acid; SC19220, 8-chloro-dibenzo[b,f][1,4]oxazepine-10(11H)-carboxylic acid, 2-acetylhydrazide; ChTX, charybdotoxin; ANOVA, analysis of variance; MΦ, macrophage; PKA, CAMP-dependent protein kinase A.
ers; and the substance(s) secreted modulate ionic current changes induced by other secretagogues and neurotransmitters such as ACh. The latter effect may contribute to hypersecretory changes during airway inflammation. We examined the effect of substances released by zymosan-activated AMs and PGE$_2$ on SGCs, measured as $\Delta I_{SC}$ across confluent SGC monolayers in Ussing chambers. Our results demonstrate that zymosan induces COX-2 expression and PGE$_2$ release by AMs. Supernatant from zymosan-activated AMs or PGE$_2$-induced $\Delta I_{SC}$ mediated via activation of CFTR chloride channels and K$^+$ channels as well as enhancing the response to ACh-induced $\Delta I_{SC}$ and K$^+$ current in SGCs. These results suggest a role for the AMs activated by particulates in stimulating airway fluid secretion.

Materials and Methods

Macrophage Culture and Drug Treatment. Male weanling pigs (Yorkshire) from a local vendor weighing ~30 kg were sacrificed by exsanguination after isoflurane anesthesia. This method was approved by the local Institutional Animal Care and Use Committee. After exsanguination, macrophages were collected by bronchoalveolar lavage using 300 ml Ca$^{2+}$- and Mg$^{2+}$-free Hanks’ balanced salt solution (137 mM NaCl, 4.2 mM NaHCO$_3$, 0.3 mM Na$_2$HPO$_4$, 5.4 mM KCl, 5.5 mM glucose, 0.5 mM EDTA, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, and 25 $\mu$g/ml kanamycin, pH 7.4, 4°C). The cells were recovered from the lavage solution by centrifugation, washed in the same lavage solution (100 g x 10 min at 4°C) three times, and then resuspended in DMEM/F-12 medium with 1% heat-inactivated fetal bovine serum and plated at a density of 5 x 10$^5$ cells/cm$^2$ in six-well culture dishes (4 ml of culture media, 9.6-cm$^2$ surface area). Cells were maintained in a humidified atmosphere containing 5% CO$_2$ at 37°C for 1 h. Unattached cells were removed by washing with phosphate-buffered saline (PBS) (3x) and culture media (1x). The estimated final cell density was 1.7 x 10$^5$ cells/cm$^2$ or 1.6 x 10$^6$ cells/ml media. Usually at least 1 to approximately 2 x 10$^6$ cells were collected by a single lavage. Greater than 98% of the cells were CD14 positive as determined by immunohistochemistry. Macrophages were activated by adding zymosan A in suspension to the culture medium (0.1 mg/ml). Indomethacin and dexamethasone were dissolved in DMSO (1000x stock solution) and added to the culture dish with or without zymosan. After 24 h of incubation, the culture medium was collected and centrifuged at 400g for 6 min at 0°C. The supernatant was frozen immediately and stored at -80°C until use. The osmolarity of macrophage culture media and supernatant was measured using a vapor pressure osmometer (model 552O; Wescor, Inc., Logan, UT).

Isolation and Culture of SGCs. Unless specifically noted, isolation and culture of SGCs were conducted according to Chan et al. (1996). After AMs were collected, the trachea was quickly removed and transported to the lab in physiological saline solution containing 140 mM NaCl, 5.5 mM KCl, 1 mM CaCl$_2$, 5.5 mM glucose, and 10 mM Hepes, pH 7.4, supplemented with penicillin and streptomycin. The epithelium was stripped off as a single layer and digested in 100 g x 10 min at 4°C) three times, and then resuspended in DMEM/F-12 medium with 1% heat-inactivated fetal bovine serum and plated at a density of 5 x 10$^5$ cells/cm$^2$ in six-well culture dishes (4 ml of culture media, 9.6-cm$^2$ surface area). Cells were maintained in a humidified atmosphere containing 5% CO$_2$ at 37°C for 1 h. Unattached cells were removed by washing with phosphate-buffered saline (PBS) (3x) and culture media (1x). The estimated final cell density was 1.7 x 10$^5$ cells/cm$^2$ or 1.6 x 10$^6$ cells/ml media. Usually at least 1 to approximately 2 x 10$^6$ cells were collected by a single lavage. Greater than 98% of the cells were CD14 positive as determined by immunohistochemistry. Macrophages were activated by adding zymosan A in suspension to the culture medium (0.1 mg/ml). Indomethacin and dexamethasone were dissolved in DMSO (1000x stock solution) and added to the culture dish with or without zymosan. After 24 h of incubation, the culture medium was collected and centrifuged at 400g for 6 min at 0°C. The supernatant was frozen immediately and stored at -80°C until use. The osmolarity of macrophage culture media and supernatant was measured using a vapor pressure osmometer (model 552O; Wescor, Inc., Logan, UT).

Measurement of Prostanoid Concentration. Radioimmunoassay of PGE$_2$ in the supernatant was performed by Dr. Jay Westcott of National Jewish Medical and Research Center (Denver, CO). DMEM/F-12 medium was used as a blank control, and supernatant from zymosan-activated AMs was diluted before performing the assay.

Protein Purification and Western Blot. To examine the COX-1 and COX-2 protein expression levels, polyclonal antibodies for COX-1 and 2 and their respective secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). After 24 h of zymosan or vehicle treatment, supernatants from AM culture were collected, and AMs were rinsed once with cold PBS.
AMs from six-well dishes treated under the same conditions were combined and lysed in 200 μl of lysis buffer (1× PBS, pH 7.4, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 10 μM/ml phenylmethylsulfonyl fluoride, 50 U/ml aprotinin, and 10 μM/ml Na4VO4) at 0°C. Cell lysates were centrifuged at 10,000g for 10 min at 4°C to yield whole-cell extracts. Protein concentration in the whole-cell extract was determined using a Coomassie protein assay kit according to manufacturer’s instructions (Pierce Chemical, Rockford, IL). Whole-cell extracts (10 μg protein/lane) were then denatured and electrophoresed into a 7.5% SDS-polyacrylamide running gel. Proteins were transferred from the gel to a nitrocellulose membrane (Bio-Rad, Hercules, CA) according to manufacturer’s instructions. Membranes were blocked for the non-specific binding by incubating in 5% milk in 1× TBS (10 mM Tris-HCl and 150 mM NaCl, pH 8.0) at 4°C overnight. Membranes were then incubated with primary antibodies diluted with 5% milk in 1× TBS (0.05% Tween 20 and 1× TBS) for 1 h at room temperature. Primary antibody concentrations for COX-2 (sc-1745, goat polyclonal) and COX-1 (sc-7950, rabbit polyclonal) were optimized at 1:2000 and 1:100 dilutions, respectively. After primary antibody incubation, the membranes were washed three times with 1× TBS for 5 min each and then incubated for 45 min at room temperature with horseradish peroxidase-conjugated secondary antibodies. Secondary antibody concentrations were optimized at 1:5000 for COX-2 (sc-2033, donkey anti-goat IgG) and 1:10,000 for COX-1 (sc-2317, donkey anti-rabbit IgG), respectively. Membranes were washed three times for 5 min each with TBS and once for 5 min with TBS. The protein bands and molecular weight markers were detected using Hyperfilm and ECL plus reagent (GE Healthcare, Little Chalfont, Buckinghamshire, UK). The protein levels and molecular weights were determined using a personal densitometer and the ImageQuant program (GE Healthcare). Band densities were normalized to the signal from AMs not exposed to zymosan and drugs.

For identification of endoprostanooid receptor proteins, polyclonal antibodies against these receptors (EP1, EP2, EP3, and EP4) were purchased from Cayman Chemical (Ann Arbor, MI). SGCs were collected using discontinuous Percoll gradient (1.5 × 10⁶/2.5 × 10⁶ cells/sample), and protein was extracted and measured as described above. Whole-cell lysates were precleared by adding 0.25 μg/ml normal rabbit or goat IgG (Santa Cruz Biotechnology, Inc.) and incubating for 30 min at 4°C. Supernatant was collected after centrifugation at 2500 rpm for 5 min. To 1 ml of supernatants, primary antibodies to EP1, EP2, EP3, or EP4 (2 μg each) were added and incubated for 1 h at 4°C. Protein A/G PLUS-Agarose (Santa Cruz Biotechnology, Inc.) was then added to each sample and after 1-h incubation, immunoprecipitates were collected by centrifugation at 3000 rpm for 5 min (Mamoon et al., 2004). After washing the pellets four times with PBS, 40 μl of electrophoresis sample buffer was added to each sample and boiled for 90 s. Proteins were separated, transferred to nitrocellulose membranes, and detected as described above.

Materials. Protein purification reagents were purchased from Santa Cruz Biotechnology, Inc. Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT). PC-1 medium containing 2 mM Glutamax and serum substitutes was purchased from Cambrex Bio Science Walkersville (Walkersville, MD). AH6809 and SC19220 were purchased from Cayman Chemical. Dexamethasone, DIDS, DMEM, DMSO, DNP, DPC, glibenclamide, indomethacin, PGE2, PGF2α, Percoll, protease, Zymosan A, and other reagents were purchased from Sigma-Aldrich (St. Louis, MO). DMSO was used to dissolve water-insoluble reagents used in Ussing chamber studies and stored at −20°C. These stocks were further diluted in Krebs-Ringers buffer containing 5% milk in 1× TBS. The final concentration of DMSO in the macrophage culture or Ussing chamber solution was equal to or less than 0.1% (v/v). Charybdoxin was dissolved in water as a 100 μM stock solution. DPC (Sigma-Aldrich) was dissolved in with 0.1 N NaOH as a 1 M stock solution.

Data Analysis. All data are expressed as mean ± S.E.M., and n values reported are the number of animals used for each experiment. To examine the relative sensitivities of SGCs to agonists under different experiment conditions, EC50 values were calculated from individual cumulative concentration-response data using sigmoid fit functions of Origin 7.0 (OriginLab Corp) and then averaged. Data from multiple treatment groups were analyzed by one-way ANOVA or one-way repeated measures ANOVA followed by the Student-Newman-Keuls pair-wise test for multiple comparisons, whenever appropriate (Sigma Stat software; SPSS Inc., Chicago, IL). In some cases, ANOVA on ranks or repeated measures ANOVA on ranks were used instead when the variances of the data were not equal among all treatment groups. Student’s t test was used to compare the data from two groups with either paired or unpaired tests as appropriate. A value of p < 0.05 was regarded as significant.

Results

The resting transepithelial potentials, currents, and resistances for confluent SGC monolayers were 5.1 ± 0.5 mV, 5.0 ± 0.7 μA/cm², and 1.8 ± 0.2 KΩ/cm², respectively, at approximately 3 to 5 days after primary culture (n = 11). At the beginning of all Ussing chamber experiments, 10 μM amiloride was added apically to block sodium absorption. This caused an average 1.0 ± 0.1 μA/cm² decrease in ΔISC ([n = 11]. Supernatant taken from 24-h zymosan-exposed AMs had average osmolarities of 320 mOsm/l and pH values of 7.2, close to values of fresh DMEM (320 and 7.4, respectively). The addition of DMEM to the Ussing chambers at up to 10% (v/v) had little effect on the total osmolarity or pH value of the Krebs-Rings buffer solution (310 mOsm/l and pH 7.4, respectively) and did not change ISC of SGC monolayers.

Effect of Supernatant from Zymosan-Activated AMs on ΔISC

To examine the direct effect of substances released by activated AMs in increasing ISC, supernatant from 24-h zymosan-exposed AMs (MΦ + zymosan supernatant) was applied cumulatively to the serosal side of the SGC monolayers. The supernatant-induced ΔISC is illustrated in Fig. 1A. A small increase in ISC was induced at 0.3% dilution (ΔISC = 1.1 ± 0.3 μA/cm²). ISC increased with increasing volume added. The ΔISC induced by 1 and 10% dilution were 3.1 ± 0.7 and 9.0 ± 0.8 μA/cm², respectively (n = 5). The increase in ISC was persistent, reaching a plateau about 5 min of exposure to MΦ + zymosan supernatant. The transmembrane resistance also decreased as shown by the increase in amplitude of the currents induced by voltage pulses (2 mV every 30 s) applied to the epithelium. The ISC increased by MΦ + zymosan supernatant was abolished by 1 mM DPC but not by 100 μM DIDS applied apically (n = 3). The concentration-response relationship of MΦ + zymosan supernatant-induced ΔISC is shown in Fig. 1D (filled squares, n = 5). A maximal response was not reached at the dilutions used (up to 10%) in these experiments. Apical addition of MΦ + zymosan supernatant (up to 10%) did not induce significant increases in ISC (n = 3). Supernatant applied serosally from 1-, 2-, 3-, 6-, and 12-h zymosan-treated AMs did not cause significant increases in ISC (data not shown). Supernatant removed from AMs incubated for 24 h without zymosan exposure (MΦ-only supernatant) applied to the serosal side of the SGC epithelium induced ΔISC of 1.4 ± 0.8 μA/cm² at 10% dilution (Fig. 4A, middle trace marked with MΦ-only, n = 3).
Supernatant from zymosan-activated AMs increased $I_{sc}$ across the confluent SGC monolayers in the Ussing chamber. A, $I_{sc}$ was recorded under voltage-clamp at 0-mV potential difference across SGC monolayers. Pulses (2 mV; every 30 s) were used to monitor conductance changes. Supernatant from zymosan-activated macrophage or control macrophage (shown in Fig. 4A, trace, Mφ-only) was added cumulatively to the serosal side of monolayers to give final dilutions marked by arrows (from 0.1–10%). The difference of $I_{sc}$ from plateau to baseline at each dilution was measured as the response to supernatant normalized to the area of the SGC monolayers (0.6 cm$^2$) as $\Delta I_{sc}$ (microamperes per centimeter squared). DIDS or DPC was added to the apical side to inhibit Cl$^-$ channels marked at arrows. D, summary of concentration-response data for Mφ + zymosan supernatant (filled squares, $n = 5$), PGE$_2$ (open squares, $n = 4$), and PGF$_{2\alpha}$ (open circles, $n = 3$) in inducing $\Delta I_{sc}$ from A and C. Data shown as mean ± S.E.M., and $n$ is the number of animals used. The dotted line shows that 100% Mφ + zymosan supernatant has an estimated equivalent potency comparable with $10^{-7}$ M PGE$_2$.

Effects of Indomethacin and Dexamethasone in Inhibiting the Production of Active Substance by Zymosan-Activated AMs. There are numerous substances released from macrophages on activation, the mix changing with the stimulus used and the length of time after activation (Lohmann-Matthes et al., 1994). The active substances produced that elicited a change in $\Delta I_{sc}$ were not observed in supernatant until more than 12 h after zymosan exposure, suggesting that release of preformed mediators was not involved. In addition, the substances produced must be fairly stable to accumulate in culture medium for 24 h at 37°C without being totally degraded. Therefore, since it has been shown that zymosan can induce the expression of cyclooxygenase (Vicente et al., 2001) and that some prostanoids, such as PGE$_2$ and PGF$_{2\alpha}$, are quite stable in culture medium, we examined the possibility that the active products were prostaglandins.

As shown in Fig. 1B, indomethacin (5 μM), a nonselective COX blocker, was added to the AM culture during 24-h zymosan exposure. Dilution (1%) of supernatant from AMs exposed to both indomethacin and zymosan did not increase $I_{sc}$ (trace 3, Mφ + Indo + zymosan supernatant), $\Delta I_{sc} = 0$ ± 0 μA/cm$^2$, whereas 1% Mφ + zymosan supernatant induced an increase in $I_{sc}$ (trace 1, Mφ + zymosan supernatant), $\Delta I_{sc} = 2.7 ± 0.3$ μA/cm$^2$ (n = 5 each, p < 0.05). Supernatant from AMs exposed to indomethacin alone had no effect on $\Delta I_{sc}$ (trace 2, Mφ + Indo supernatant, $n = 5$). Residual indomethacin in the supernatant had no direct effect on the SGCs since application of equivalent dilution of indomethacin (50 nM, $n = 5$) directly to the Ussing chamber did not change the response of SGCs to Mφ + zymosan supernatant (data not shown).

We also treated AM culture with 1 μM dexamethasone, a known suppressor of macrophage function (Becker and Grasso, 1985). Supernatant from AMs treated with dexamethasone (1%) during 24-h zymosan exposure induced significantly less $\Delta I_{sc}$ compared with $\Delta I_{sc}$ induced by 1% Mφ + zymosan supernatant. Dexamethasone treatment caused a 70.5 ± 2.4% inhibition of $\Delta I_{sc}$ compared with Mφ + zymosan supernatant ($n = 3$, p < 0.05). There was no direct action of the residual dexamethasone in the supernatant on the SGCs since application of equivalent dilution of dexamethasone (10 nM, $n = 3$) directly to the Ussing chamber had no effect on the SGC response to Mφ + zymosan supernatant (data not shown). DMSO vehicle (0.1% dilution) in macrophage culture did not influence the supernatant-induced $\Delta I_{sc}$. 

Fig. 1. Supernatant from zymosan-activated AMs increased $I_{sc}$ across the confluent SGC monolayers in the Ussing chamber. A, $I_{sc}$ was recorded under voltage-clamp at 0-mV potential difference across SGC monolayers. Pulses (2 mV; every 30 s) were used to monitor conductance changes. Supernatant from zymosan-activated macrophage or control macrophage (shown in Fig. 4A, trace, Mφ-only) was added cumulatively to the serosal side of monolayers to give final dilutions marked by arrows (from 0.1–10%). The difference of $I_{sc}$ from plateau to baseline at each dilution was measured as the response to supernatant normalized to the area of the SGC monolayers (0.6 cm$^2$) as $\Delta I_{sc}$ (microamperes per centimeter squared). DIDS or DPC was added to the apical side to inhibit Cl$^-$ channels marked at arrows. B, indomethacin (5 μM) was added to macrophage cultures during 24-h zymosan exposure. Indomethacin treatment abolished the increase in $I_{sc}$ induced by supernatant (trace 3, Mφ + Indo + zymosan supernatant, $n = 5$). Residual indomethacin (~50 nM) in the Ussing chamber originating from supernatant did not affecting $I_{sc}$ (trace 2, Mφ + Indo supernatant, $n = 5$). C, PGE$_2$ or PGF$_{2\alpha}$ was applied serosally to the SGC monolayers from 3 × 10$^{-11}$ to 10$^{-5}$ M concentrations (cumulative), which induced persistent increases in $I_{sc}$.
The Comparison of Prostaglandins and Supernatant in Inducing $\Delta I_{\text{ ISC}}$. The ability of prostaglandins to increase $I_{\text{ ISC}}$ of SGC was examined. In Fig. 1C, PGE$_2$ or PGF$_2\alpha$ applied cumulatively to the serosal side of SGC monolayers increased $I_{\text{ ISC}}$. $I_{\text{ ISC}}$ rises to a stable plateau within 5 min after the agonist application, similar to the response to M$\phi$ + zymosan supernatant. PGE$_2$-induced $\Delta I_{\text{ ISC}}$ reach a maximum at approximately $10^{-6}$ M in the case of PGE$_2$ (Fig. 1D, open squares, maximal $\Delta I_{\text{ ISC}} = 15.7 \pm 1.4 \mu A/cm^2$, $n = 4$). The EC$_{50}$ for serosal PGE$_2$-induced $\Delta I_{\text{ ISC}}$ is 10.0 $\pm$ 1.8 nM ($n = 4$). Serosal PGF$_2\alpha$ was less potent than PGE$_2$ in inducing $\Delta I_{\text{ ISC}}$, with an estimated EC$_{50}$ of 1.1 $\pm$ 0.1 $\mu$M (Fig. 1D, $\Delta I_{\text{ ISC}} = 6.4 \pm 0.7 \mu A/cm^2$ at $10^{-5}$ M, open circles, $n = 3$). We also applied another prostaglandin, PGD$_2$, to the serosal side of SGC monolayer, which induced $\Delta I_{\text{ ISC}}$ with an EC$_{50}$ of $\sim 19$ nM (one experiment, data not shown). As also shown in the Fig. 1D, 100% M$\phi$ + zymosan supernatant is estimated to induce $\Delta I_{\text{ ISC}}$ comparable with that induced by $10^{-7}$ M PGE$_2$.

PGE$_2$ Concentration in the Supernatant of Zymosan-Activated AMs. PGE$_2$ levels in M$\phi$-only supernatant measured using radioimmunoassay was 10 $\pm$ 1 ng/ml ($\sim 28 \pm 3$ nM, $n = 3$). The PGE$_2$ concentration reached 195 $\pm$ 28 ng/ml ($\sim 550 \pm 79$ nM, $n = 3$) in M$\phi$ + zymosan supernatant, an approximate 20-fold increase in PGE$_2$ release into the culture medium compared with AMs not exposed to zymosan ($p < 0.05$). PGE$_2$ was not detectable in fresh culture media.

Cyclooxygenase Expression and Zymosan Exposure of AMs. The delay in production of active substance in inducing $\Delta I_{\text{ ISC}}$ is consistent with increased expression of a protein. We examined the effects of zymosan exposure on COX-1 and COX-2 expression levels in AMs. As shown in Fig. 2, Western blots for both COX-1 and COX-2 yielded single bands with estimated molecular masses of approximately 79 to 90 kDa. COX-1 levels, as estimated using Western blot (Fig. 2A, A and inset), were not affected by zymosan exposure for 24 h (98 $\pm$ 13% of basal level, $n = 3$). Indomethacin (5 $\mu$M) or dexamethasone (1 $\mu$M) present during zymosan exposure did not alter COX-1 expression levels (Fig. 2A, bar graph, 153 $\pm$ 17% and 122 $\pm$ 29% of basal level, respectively, $n = 3$ each). COX-1 expression levels were not significantly different among all treatment groups ($p > 0.05$).

COX-2 expression levels were increased as early as 6 h but markedly increased by 24-h zymosan exposure (data not shown). As shown in the Western blot and bar graph in Fig. 2, B and inset, zymosan exposure (24 h) significantly increased COX-2 expression levels in nondrug, 5 $\mu$M indomethacin-, and 1 $\mu$M dexamethasone-treated groups (filled bars for zymosan-treated AMs versus empty bars for nonzymosan-treated AMs, $n = 3$, $p < 0.05$). Zymosan exposure increased COX-2 expression level to 540 $\pm$ 100% of basal level in nondrug and nonzymosan-treated AMs, to 850 $\pm$ 200% in indomethacin- and zymosan-treated AMs (versus 220 $\pm$ 50% in AMs treated with indomethacin alone), and to 120 $\pm$ 20% in dexamethasone- and zymosan-treated AMs (versus 20 $\pm$ 10% in macrophage treated with dexamethasone alone). The presence of dexamethasone during zymosan exposure significantly suppressed the expression of COX-2 (120 $\pm$ 20% of basal level versus 540 $\pm$ 100% of basal level in zymosan-treated AMs with and without dexamethasone, respectively, $p < 0.05$). Indomethacin (5 $\mu$M) treatment alone significantly increased COX-2 expression to 225 $\pm$ 52% of basal level ($p < 0.05$).
PGE₂ was $3.6 \pm 1.8 \mu M$ ($n = 3$, Fig. 3B, circles), significantly higher than that for serosal PGE₂ application in parallel experiments ($EC_{50} = 15.5 \pm 1.3 \text{nM}$, $n = 4$, squares, $p < 0.05$ using Student’s $t$ test).

PGE₂ is less potent in inducing ISC in collagenase-dissociated SGCs than protease-dissociated SGCs ($167 \pm 34 \text{nM}$, $n = 4$ versus $10.0 \pm 1.8 \text{nM}$, $n = 4$ in Fig. 1D or $15.5 \pm 1.3 \text{nM}$ in Fig. 3B, $n = 4$; $p < 0.05$). PGE₂ had similar $EC_{50}$ values in two separate protease-dissociated SGC groups shown in Figs. 1D and 3B ($p > 0.05$).

Receptors Responsible for PGE₂-Induced ΔISC. We examined the types of EP receptor involved in the PGE₂ actions. As shown in Fig. 3C, control trace, PGE₂ increased $I_{SC}$ with an estimated $EC_{50}$ of $167 \pm 34 \text{nM}$ (Fig. 3D, circles, $n = 4$). When 10 μM SC19220, an EP₁ antagonist, or 30 μM AH6809, an EP₁ and EP₂ antagonist, was applied to the serosal side of SGC monolayers, AH6809 caused right shift of the concentration-response relationship of PGE₂-induced ΔISC (Fig. 3D, upward triangles, $EC_{50} = 666 \pm 162 \text{nM}$, $n = 3$), but SC19220 did not (Fig. 3D, squares, $EC_{50} = 103 \pm 33 \text{nM}$, $n = 3$). $EC_{50}$ for AH6809-treated group was significantly different from the control and SC19220-treated groups ($p < 0.05$).

Figure 3E shows that all four EP receptor subtypes (EP₁–4) were detected using immunoprecipitation and Western blot methods in SGCs isolated with protease or collagenase-dissociated fresh SGCs, with estimated molecular masses being 42, 52, 53, and 65 kDa for EP₁, EP₂, EP₃, and EP₄ subtypes, respectively. Data points are shown as mean ± S.E.M., and $n$ is the number of animals used in the experiment.

Figure 3A shows the difference in the serosal and apical PGE₂ sensitivities and the role of EP₁ and EP₂ receptors in PGE₂-induced ΔISC in SGC monolayers. A, PGE₂ was applied cumulatively from $3 \times 10^{-11}$ to $10^{-4} \text{M}$ to the serosal side (trace 1) or apical side (trace 2) of SGC monolayers in the Ussing chamber. B, concentration-response relationships (normalized to maximal serosal response) for PGE₂-induced ΔISC are shown. The estimated $EC_{50}$ for serosal PGE₂ was $15.5 \pm 1.3 \text{nM}$ (squares, $n = 4$) and $3.6 \pm 1.8 \mu M$ (circles, $n = 3$) for apical PGE₂. C, inhibition of PGE₂-induced ΔISC by EP receptor antagonists. PGE₂ applied cumulatively from $3 \times 10^{-8}$ to $10^{-4} \text{M}$ concentrations to the serosal side of SGC monolayers induced similar response (control trace) to those shown in Figs. 1C and 1A. In parallel inserts, SC19220 (10 μM) and AH6809 (30 μM) were applied serosally prior to PGE₂ application. D, PGE₂-induced ΔISC and its inhibition by SC19220 and AH6809. Control SGC has an $EC_{50}$ of $167 \pm 34 \text{nM}$ (circles, $n = 4$) for PGE₂-induced ΔISC. SC19220-treated SGCs had an $EC_{50}$ of $103 \pm 33 \text{nM}$ (squares, $n = 3$), and AH6809-treated SGCs had an $EC_{50}$ of $666 \pm 162 \text{nM}$ (upward triangles, $n = 3$). E, immunoprecipitation and Western blot indicated the presence of all four subtypes of endoprostanoïd receptors in SGCs dissociated with either protease or collagenase (EP₁–4, approximately three to five animals were used in each group), with estimated molecular masses being 42, 52, 53, and 65 kDa for EP₁, EP₂, EP₃, and EP₄ subtypes, respectively. Data points are shown as mean ± S.E.M., and $n$ is the number of animals used in the experiment.
However, the EC50 for ACh-induced increases in ISC in SGCs not treated with supernatants (trace, control) were used as control. B, concentration-response relationships for ACh-induced ΔISC in SGCs pretreated with Mφ + zymosan supernatant (upward triangles, n = 3) and Mφ-only supernatant (squares, n = 3) and in control SGCs (circles, n = 6). C, in the bottom trace (Control), DMSO vehicle applied before ACh treatment had no effect on the responsiveness to ACh. The top two current recordings show that, 5 min prior to ACh addition, 10−6 M PGE2 or 5 × 10−6 M forskolin caused stable increases in ISC. Notice that PGE2 or forskolin pretreatment sensitized SGC monolayers to low ACh concentrations (10−8 to approximately 3 × 10−8 M) to increase ISC. D, effect of forskolin (5 × 10−6 M, squares, n = 4) or PGE2 (10−7 M, upward triangles, n = 10) pretreatment on the concentration responses for ACh-induced ΔISC (normalized to the maximal ACh-induced ΔISC response in Control SGC monolayers, circles, n = 6). Data are expressed as mean ± S.E.M., and the number of animals used in each group is indicated in the figure.

We further examined the effect of PGE2 or forskolin pretreatment on ACh-induced ΔISC. SGC monolayers were pretreated with PGE2 or forskolin (5 × 10−6 M, to elevating cytosolic cAMP level) serosally for 5 min before ACh application. Both PGE2 and forskolin induced persistent increases in ΔISC in SGCs, with average values being 140 ± 6% and 122 ± 4% of the ACh-induced maximal ΔISC in control SGCs run in parallel (p < 0.05).
response to 121 ± 20 nM (n = 10) and 84 ± 19 nM (n = 4) (Fig. 4D, top triangles and squares, respectively), whereas the EC_{50} for ACh-induced ΔISC in controls was 418 ± 28 nM (Fig. 4D, circles, n = 12). The shift in the sensitivity to ACh depended on the concentration of PGE_2. An increase in sensitivity to ACh could be detected at 10^{-8} M PGE_2 and reached maximum at 10^{-6} M PGE_2. PGE_2 pretreatment shifted the EC_{50} for ACh-induced ΔISC to 292 ± 37 (n = 3), 188 ± 55 (n = 3), and 103 ± 35 (n = 3) nM at 10^{-9}, 10^{-8}, and 10^{-6} M PGE_2 concentrations, respectively. The EC_{50} values for PGE_2- (from 10^{-8} M and up) and forskolin-pretreated groups were significantly different from control (p < 0.01).

Pretreatment with 10^{-7}, 10^{-6} PGE_2, or 5 × 10^{-6} M forskolin had similar effects in sensitizing ACh-induced ΔISC, and the EC_{50} values for these three groups were not significantly different from each other (p > 0.05).

PGE_2 (10^{-5} M) was also applied serosally before ACh application. The EC_{50} was shifted for the ACh-induced ΔISC to 87 ± 8 nM (n = 3), significantly different from that in controls (418 ± 28 nM, n = 12, p < 0.05).

The ACh-induced maximal ΔISC in groups treated with 10^{-8}, 10^{-7}, and 10^{-6} M PGE_2 were significantly greater than control monolayers tested in parallel, the average increases being 143 ± 6% (n = 3), 135 ± 5% (n = 3), and 131 ± 7% (n = 3) of ACh-induced maximal ΔISC in control (p < 0.05), respectively. ACh-induced maximal ΔISC in SGCs treated with 10^{-9} M PGE_2 or 5 × 10^{-6} M forskolin were not significantly different from those of control, the average values being 104 ± 9% and 107 ± 8% of control, respectively (p > 0.05).

**K^+ and Cl^- Channels Involved in PGE_2- or ACh-Induced ΔISC.** ChTX, a K_{V} blocker, and chromanol 293B, a blocker of K_{V}LQT1 (KCNQ1)/KCNE3 K^+ channels (Lohmann et al., 1995), were used to examine the involvement of these K^+ channels in the PGE_2- and ACh-induced increases in ΔISC. Previous reports showed that the K_{V}LQT1 (KCNQ1) are present in human bronchial epithelial cells and the Calu-3 serous cell line, K_{V}LQT1 are involved in cAMP-mediated Cl^- secretion (Mall et al., 2000; Cowley and Linsdell, 2002). Chromanol 293B, at 200 μM (supramaximal concentration), was applied serosally to SGC monolayers after 10^{-7} M PGE_2 but before ACh applications, which caused an average 63 ± 3% decrease in PGE_2-induced ΔISC (Fig. 5A, trace 3, n = 4). However, chromanol 293B did not change the amplitude or potency of ACh-induced increase in ΔISC. ACh-induced maximal ΔISC in PGE_2 + 293B-treated group was 108 ± 13% of PGE_2 treatment group (Fig. 5B, p > 0.05). Chromanol 293B treatment did not alter the PGE_2-induced reduction in EC_{50} of ACh-induced ΔISC (Fig. 5B, circles, 123 ± 36 nM for PGE_2 treatment group versus 191 ± 82 nM for PGE_2 + 293B treatment group, top triangles, n = 4 each, p > 0.05).

ChTX (100 nM) was added serosally after 10^{-7} PGE_2 application and blocked 24 ± 4% (n = 4) of 10^{-7} M PGE_2-induced ΔISC. ChTX also blocked 66 ± 1% of ACh-induced maximal ΔISC compared with that in SGCs not treated with ChTX (Fig. 5, A, trace 2, and B, squares, n = 4). EC_{50} values for ACh-induced ΔISC were 171 ± 37 and 109 ± 26 nM for SGC monolayers treated with and without 100 nM ChTX, respectively (n = 4 each, p > 0.05). All these SGC monolayers were pretreated with 10^{-7} M PGE_2. The effect of K^+ channel inhibition on the ACh concentration-response relationships for PGE_2-pretreated SGCs is shown in Fig. 5B. In SGC monolayers not treated with PGE_2, ChTX (100 nM) added before ACh actions also blocked 60 ± 7% of ACh-induced maximal ΔISC (n = 4).

![Fig. 5. PGE_2- and ACh-induced ΔISC were blocked by K^+ and Cl^- channel blockers.](image-url)
To test the possibility that PGE₂ activates CFTR channels and facilitates the ACh response, 2 mM (maximum concentration) glibenclamide was applied apically to the SGC monolayers to block CFTR channel activity. Glibenclamide, a relatively nonspecific CFTR channel blocker, has been used to study the role of CFTR in the epithelial cells (Krouse et al., 2004). Glibenclamide abolished 10⁻⁷ M PGE₂-induced increase in I_{SC} to below baseline levels (Fig. 5C, n = 3). After PGE₂ and glibenclamide treatment, ACh-induced increases in I_{SC} were also inhibited (Fig. 5D), a partial enlarged view of 5C, averaged maximal ΔI_{SC} was −1 μA/cm², n = 3). The EC₅₀ of ACh-induced increases in I_{SC} after PGE₂ and glibenclamide treatment was 182 ± 28 nM (n = 3), which was not significantly different from control (123 ± 36 nM, n = 4, p > 0.05). These data were consistent with results that supernatant- and PGE₂-induced increases in I_{SC} were abolished by apically applied 1 mM DPC but less affected by 100 μM DIDS (Fig. 1A).

Effect of PGE₂ on the ACh-Induced Serosal K⁺ Current. To study the modulation of ACh-induced increases in serosal K⁺ current by PGE₂, we used 180 μg/ml nystatin to permeabilize the apical membrane of the SGC monolayers to small monovalent ions, replaced Cl⁻ in the solution with gluconate ion, and established a high apical to serosal K⁺ concentration gradient as described under Materials and Methods. About 10 min after addition of nystatin, basal I_{SC} increased and then stabilized an indication of successful permeabilization of the apical membrane (Fig. 6A). ACh addition increased serosal K⁺ current with an EC₅₀ of 720 ± 111 nM (Fig. 6B, circles, n = 3). Pretreating permeabilized SGC monolayers with 10⁻⁷ M PGE₂ prior to ACh application increased ΔI_{SC} (3.6 ± 1.1 μA/cm², n = 3) and significantly sensitized ACh-induced increases in K⁺ current (EC₅₀ = 185 ± 52 nM, Fig. 6B, upward triangles, p < 0.05, n = 3). No enhancement of ACh-induced maximal K⁺ current by PGE₂ pretreatment occurred, and ACh-induced maximal K⁺ current was 82 ± 5% of control (p > 0.05, n = 3).

Discussion

The effects of AM-released substances in stimulating and enhancing increases in I_{SC} in the SGC monolayers were examined. Zymosan activated AMs by inducing the expression of COX-2 and the production of PGE₂. Supernatant from activated AMs or prostanoids increased I_{SC} in SGC monolayers. Pretreatment of SGC monolayers with supernatant from activated AMs, PGE₂, or forskolin enhanced ACh-induced ΔI_{SC}. Our findings suggest that prostanoids produced by AMs enhance SGC secretory functions.

Unopsonized zymosan activates naive macrophages via the mannose receptor and Toll-like receptors to initiate innate immune responses (Takeuchi and Akira, 2001), leading to phagocytosis, arachidonic acid release, and COX-catalyzed prostanoid productions (Girotti et al., 2004), as well as cyto¬kin production (Lohmann-Matthes et al., 1994). In this study, it took more than 12 h for sufficient amount of active substances to increase ΔI_{SC} to appear in the supernatant, suggesting that protein synthesis was required. COX-2 expression was increased at 6 to 24 h after zymosan exposure, an effect blunted by glucocorticoid exposure. The ability of zymosan-activated AMs supernatant to increase I_{SC} was blocked by indomethacin and reduced by dexamethasone, suggesting that the active components of supernatant were COX-related prostanoids. PGE₂ accumulated in the supernatant at a concentration of ~5 × 10⁻⁷ M after 1.6 × 10⁶/ml AMs were exposed to 0.1 mg/ml zymosan for 24 h. At this concentration, PGE₂ induces significant ΔI_{SC} when applied serosally (Fig. 1, C and D). About 2 × 10⁶ AMs were recovered by a single lavage of the lungs, we estimated that if this number of AMs were to be activated in vivo to the same degree as in vitro, 5 × 10⁻⁷ M PGE₂ could be reached in >100 ml of airway fluid.

Products released by AMs in the lumen are secreted onto the apical surface of the epithelium. In Calu-3 cells, a cell line with both serous and mucus gland cell properties (Shen et al., 1994), isoprostane 8-iso-prostaglandin E₂ induced ΔI_{SC} with apical EC₅₀ higher than serosal EC₅₀ (Cowley, 2003). In this study, PGE₂ applied apically significantly induced ΔI_{SC} at 10⁻⁷ M, but its EC₅₀ was significantly higher than that for serosal application (3.6 μM versus 15.5 nM, Fig. 3, A and B). Supernatant applied apically (10% dilution) did not induce significant ΔI_{SC}, but applying undiluted supernatant would induce significant ΔI_{SC}, comparable with 10⁻⁷ M PGE₂ (Fig.

Fig. 6. PGE₂-sensitized ACh-induced serosal K⁺ current in nystatin-permeabilized SGC monolayers. A, recording of K⁺ current in response to PGE₂ and ACh. The apical membrane of SGC monolayers was permeabilized with 180 μg/ml nystatin (at first arrow), causing a stable increase in current. NaCl was replaced with potassium gluconate and sodium gluconate in the apical and serosal solutions, respectively. PGE₂ (10⁻⁷ M) was added serosally 5 min before ACh treatment (gray trace). The black trace is a control recording without PGE₂ pretreatment. Cumulative concentration-response relationships for ACh-induced K⁺ current were then generated. B, average concentration-response relationships for ACh-induced K⁺ current (calculated as percentage of ACh-induced maximal response in control). Data are expressed as mean ± S.E.M., and the number of animals used in each group is indicated in the figure.
Although PGE$_2$ is poorly metabolized in cell culture, it has a half-life of less than 30 s in the circulation (Camus and Jeannin, 1984); thus, PGE$_2$ is an autocrine or paracrine hormone acting locally to stimulate airway secretion when released into the surface airway liquid. In vivo, products secreted by activated AMs cross the epithelium to induce both local and systemic actions. Lung instillation of products from cocultures of epithelial cells and macrophages exposed to PM10 causes bone marrow stimulation in rabbits (Fuji et al., 2002). Inhaling particulates induces airway hyperreactivity (Walters et al., 2001) and has systemic inflammatory effects (van Eeden and Hogg, 2002). Kitano et al. (1992) showed that intact airway constricted and secreted mucus after intraluminal application of methacholine, although the EC$_{50}$ was significantly higher than that for serosal application. Relatively lipophilic compounds in supernatant from activated AMs, such as PGE$_2$, should cross the epithelium to affect underlying tissues by activating serosal receptors, although we cannot rule out specific receptors in the apical cell process.

PGE$_2$ binds to four EP receptor types (EP$_{1-4}$). All are present in SGCs (Fig. 3E). EP$_{1,3}$ receptors are linked to phospholipase C, and EP$_{2,4}$ receptors are linked to adenyl cyclase (Breyer et al., 2001). In SGCs, supernant- and PGE$_2$- or forskolin-induced stable $\Delta$I$_{SC}$ similar to cAMP-elevating agent-induced $\Delta$I$_{SC}$ in Calu-3 cells (Cowley, 2003) by activation of CFTR since both supernatant- and PGE$_2$-induced $\Delta$I$_{SC}$ were blocked by apically applied DPC or glibenclamide but less by DIDS. In addition, a cAMP-activated K$^+$ channel (K$_{LQT1}$) is also involved since PGE$_2$-induced $\Delta$I$_{SC}$ were reduced by serosally applied chromanol 293B. The preferential inhibition by the EP$_{1,2}$ receptor antagonist AH6809, but not by the EP$_{1}$ receptor antagonist SC19220, suggests that the EP$_{2}$ receptor induces $\Delta$I$_{SC}$ to PGE$_2$.

Unlike PGE$_{2}$, ACh induced transient $\Delta$I$_{SC}$ followed by plateaus. ACh belongs to Ca$^{2+}$-elevating neurotransmitters released by parasympathetic nerves (Coulson and Fryer, 2003). ACh activates $\beta_{2}$ receptors to increase $I_{LSC}$ (Liu and Farley, 2005) and to initiate fluid secretion via SGCs (Yang et al., 1988; Ishihara et al., 1992). The increase in $I_{LSC}$ is most likely brought about through production of inositol 1,4,5-trisphosphate and Ca$^{2+}$ release from internal stores. Ca$^{2+}$ not only activates K$_{Ca}$, which induces membrane hyperpolarization and net flux of Cl$^-$/HCO$_3^-$ into the lumen (Ballard and Inglis, 2004) but also stimulates mucus secretion (Ishihara et al., 1992). ACh-induced $\Delta$I$_{SC}$ were significantly blunted by ChTX, suggesting the ACh response depended on the K$_{Ca}$. An intermediate conductance K$_{Ca}$ has been found in Calu-3 cells (Cowley and Lindsell, 2002). The cAMP-activated K$_{LQT1}$ K$^+$ channels are not likely to be involved in the ACh-induced $\Delta$I$_{SC}$ since the latter was not affected by chromanol 293B.

In SGCs, there are two cell types with characteristics consistent with serous and mucus cells. Serous cells and the Calu-3 cells respond to cAMP-elevating agents with increases in both CFTR and K$_{LQT1}$ K$^+$ currents, the net effect being HCO$_3^-$/Cl$^-$ exit through CFTR. In Calu-3 cells, muscarinic activation also induces membrane hyperpolarization and net flux of Cl$^-$/HCO$_3^-$ (Ballard and Inglis, 2004). Mucus gland cells have fewer CFTRs, and cAMP-elevating agents do not induce significant ionic current (Tamada et al., 2000). Our previous data showed that SGCs isolated using discontinuous Percoll gradients (Yang et al., 1991; Chan et al., 1996) consist of about 70% mucus cells and 30% serous cells using periodic acid-Schiff and Alcian Blue staining methods. Thus, $\Delta$I$_{SC}$ in Ussing chamber measurements are combination of ionic currents mediated by serous and mucus gland cells. Farley et al. (1991) reported that the magnitude of ACh-induced $\Delta$I$_{SC}$ was independent of isoproterenol-induced $\Delta$I$_{SC}$ in isolated tracheal epithelium preparations at supramaximal drug concentrations (10$^{-5}$ M each). They concluded that isoproterenol and ACh responses occurred in different cell types, presumably serous and mucus cells. In this experiment, supernatant from zymosan-activated AMs, PGE$_2$, or forskolin shifted the concentration-response relationships for the ACh-induced $\Delta$I$_{SC}$ to the left relative to untreated controls resulting in a greater than 3-fold increase in apparent sensitivity to ACh. ACh-induced maximal $\Delta$I$_{SC}$ were increased by supernatant or PGE$_2$ pretreatment. Forskolin pretreatment did not increase ACh-induced maximal $\Delta$I$_{SC}$, similar to the effect of isoproterenol on ACh response in isolated tracheal epithelium as reported by Farley et al. (1991). Therefore, PGE$_2$ or supernatant from zymosan-activated AMs increases cytosolic cAMP concentration to enhance the apparent sensitivity to ACh but may activate another pathway, resulting in an increased maximal response to ACh. The increase in maximal response to ACh may not involve increased maximal activation of serosal K$^+$ channels since the amplitude of ACh-induced serosal K$^+$ current in nystatin permeabilized SGCs was not influenced by PGE$_2$ (Fig. 6). PGE$_2$ is known to activate tyrosine kinase/phosphatidylinositol 3 kinase via the EP$_{2}$ receptor, in addition to activation of PKA (Regan, 2003), but whether this pathway is activated in SGCs is not known. EP$_{2}$ receptors are present in isolated SGCs (Fig. 3E).

The apparent increase in sensitivity to ACh occurred rapidly after exposure to PGE$_2$ and is therefore probably not due to an increased expression of receptors. Also, PGE$_2$ sensitized SGCs to histamine-induced $\Delta$I$_{SC}$ (H. Liu and J. M. Farley, unpublished data) that are mediated via $\alpha_2$ receptors (Liu and Farley, 2005). It seems likely that steps common to both ACh and histamine transduction pathways are enhanced by PGE$_2$. One common event may be the sensitization of ion channels by PKA-mediated phosphorylation. PKA reportedly sensitizes large-conductance Ca$^{2+}$-activated K$^+$ channels to Ca$^{2+}$ (Tian et al., 2001). PGE$_2$ pretreatment apparently sensitized ACh-induced serosal K$^+$ current without enhancing its maximal response (Fig. 6, A and B). ChTX reduced the ACh-induced maximal $\Delta$I$_{SC}$ without changing the apparent sensitization of ACh response by PGE$_2$ treatment (Fig. 5B). Thus, PGE$_2$-induced direct sensitization of ChTX-sensitive K$_{Ca}$ is not solely responsible for the apparent sensitization of SGCs to ACh. Chromanol 293B-sensitive cAMP-activated K$^+$ channels are not involved in such sensitization either because 293B had no effect on the ACh-induced $\Delta$I$_{SC}$ in both sensitivity and magnitude, although it inhibited the PGE$_{2}$-induced $\Delta$I$_{SC}$ (Fig. 5B).

CFTR channels are reported to be the exclusive Cl$^-$ conductance in Calu-3 cells for cholinergically mediated gland secretions (Moon et al., 1997). Our data show that glibenclamide applied apically reduced both PGE$_{2}$- and ACh-induced $\Delta$I$_{SC}$. However, Joo et al. (2002) demonstrated that cholinergic-stimulated fluid secretion occurred in tracheal/bronchial epithelium from cystic fibrosis patients, although cAMP-induced secretion did not. It is possible that CFTR loss
in serous cells is compensated for partially by Ca\(^{2+}\)–activated Cl\(^{-}\) channels in mucus cells (Ballard and Inglis, 2004). Glibenclamide may also have nonspecific effects, blocking important ion transporters essential for ACh-induced \(\Delta IS\) (Ballard and Inglis, 2004). Although glibenclamide almost completely blocked PGE\(_2\)– and ACh-induced \(\Delta IS\), the sensitization of the EC\(_{50}\) for ACh-induced \(\Delta IS\) was not affected by glibenclamide (Fig. 5D). These data suggest that the apparent sensitization of SGCs to ACh by PGE\(_2\) is not due to the direct sensitization of ion channels such as CFTR and K\(_{ca}\) but rather through effects on the signal transduction pathway activating ion channels, presumably by enhancing the elevation of intracellular Ca\(^{2+}\).

Overall implications of this study are that exposure to particulates induces the release of PGE\(_2\) from AMs, which have significant effects on the mucosa of the airway. The products released increase ion flux (and therefore secretion of fluid) into the airway and sensitize the SGCs to respond to secretagogues. Inhibition of CFTR and K\(_{ca}\) changed the magnitude of ACh-induced response without affecting PGE\(_2\)-induced sensitization to ACh. If PGE\(_2\)-induced sensitization of SGCs to ACh was due to enhanced Ca\(^{2+}\) mobilization, we suggest that events activated by Ca\(^{2+}\), such as ACh-induced mucus release, would also be enhanced by PGE\(_2\) even if CFTR function was lost. PGE\(_2\) generally has been considered anti-inflammatory in the lung (Vancheri et al., 2004). Our study suggests that PGE\(_2\)-induced enhancement of SGC secretion response to ACh may constitute a protective mechanism during acute exposure of airway to particulates by aiding in particulate clearance; however, it may also lead to excessive fluid/mucus secretion and therefore exacerbate pathological conditions found in asthma, cystic fibrosis, or chronic obstructive pulmonary disease.

References