G protein-coupled prostaglandin receptor modulates conductive Na\(^{+}\) uptake in lung apical membrane vesicles

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Mukhopadhyay, Somnath, Asim K. Dutta-Roy, Gregor K. FYFE, Richard E. Olver, and Paul J. Kemp. G protein-coupled prostaglandin receptor modulates conductive Na\(^{+}\) uptake in lung apical membrane vesicles. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L567–L572, 1998.—Because G protein-regulated cation channels in type II pneumocytes constitute the most likely pathway for alveolar Na\(^{+}\) entry, we explored the hypothesis that a G protein-coupled prostaglandin (PG) E\(_2\) receptor controls perinatal lung alveolar Na\(^{+}\) transport. [\(^{3}H\)]PGE\(_2\) binding to the alveolar apical membrane was trypsin sensitive and showed a rank order of competitive inhibition: PGE\(_{2}\) = PGE\(_{1}\) > PGD\(_{2}\) > PGF\(_{2\alpha}\). Kinetic analysis demonstrated both high-affinity (dissociation constant (K\(_{D}\)) = 2.1 ± 0.7 nM; maximal binding (B\(_{\text{max}}\)) = 27 ± 7 fmoI/mg protein) and low-affinity (K\(_{D}\) = 28 ± 2 nM; B\(_{\text{max}}\) = 265 ± 29 fmoI/mg protein) binding sites. Modulation of high-affinity GTPase activity identified a similar potency order (IC\(_{50}\) = 11 nM for PGF\(_{2\alpha}\), vs. 10–50 \(\mu\)M for other PGs), suggesting that the receptors are G protein coupled. Finally, 1 \(\mu\)M PGE\(_{2}\) (IC\(_{50}\)) increased conductive 22Na\(^{+}\) uptake into membrane vesicles only in the presence of 100 \(\mu\)M intravesicular GTP. The K\(_{D}\) value for the high-affinity binding site together with the rank order of PG effect on ligand binding and G protein function places this PG receptor in the EP\(_{3}\) subtype, whereas Na\(^{+}\) uptake studies suggest that it helps maintain perinatal lung Na\(^{+}\) homeostasis.

sodium channel; prostaglandin E\(_{2}\); sodium ion

EFFICIENT LUNG ALVEOLAR Na\(^{+}\)-channel function is crucial to survival. Epithelial Na\(^{+}\)-channel "knockout" mice die of severe respiratory distress within 48 h of birth, with autopsy evidence of massive lung fluid overload (12). In humans, life-threatening neonatal respiratory distress commonly associates with lung alveolar and interstitial edema (2), implicating an underlying dysfunction of extra- or intracellular mechanisms regulating lung alveolar Na\(^{+}\) transport around the time of birth. After the characterization of the hormonal control (1) of this amiloride-inhibitable Na\(^{+}\)-absorptive response (24), it is now imperative to understand the cellular mechanisms that regulate this absorptive process in order to work toward the development of therapeutic strategies for the treatment of lung alveolar fluid overload in the neonate.

Working toward this objective, we have recently identified low-conductance, amiloride-sensitive, Na\(^{+}\)-selective channels in the freshly isolated mature distal alveolar (term) fetal type II pneumocyte, which we propose may facilitate this Na\(^{+}\)-driven fluid-absorptive role (8, 18). These channels are upregulated by polyunsaturated fatty acids and by the nonhydrolyzable GTP analog guanosine 5'-O-(3-thiotriphosphate) (GTP\(_{\gamma}\)S; see Ref. 8a), implicating control via a G protein-linked pathway.

Although most available information supports the hypothesis that prostaglandins (PGs) play an important role in the regulation of ion transport in the first-trimester fetal lung by stimulating fluid secretion (4, 15, 19, 32), their effect on the fetal lung in late gestation has not been studied. However, evidence from the renal collecting tubule (an Na\(^{+}\)-absorbing epithelium) would suggest that PGE\(_{2}\) is a likely candidate for control of alveolar fluid absorption. Using perfused, microdissected rabbit renal collecting tubule and adjacent thick ascending limb (containing cation channels electrophysiologically similar to perinatal mammalian lung; see Ref. 17), Grantham and Orloff (10) demonstrated a dual role for PGE\(_{2}\) in regulating Na\(^{+}\)-linked water absorption in the collecting tubule and thick ascending limb: at low concentrations (10\(^{-9}\) M), PGE\(_{2}\) inhibited, whereas at relatively higher concentrations (10\(^{-7}\) M), it stimulated the arginine vasopressin-mediated water-absorptive response. Higher concentrations of PGE\(_{2}\) (10\(^{-7}\) M) also caused stimulation of adenyl cyclase activity in both collecting tubule and thick limb cells (28). Collating this and related information, Smith (26) developed a unifying biochemical paradigm for the role of PGE\(_{2}\) in renal fluid transport.

Although PG receptors have been identified in the airway (6, 13), their existence in lung alveoli (with different functional priorities such as surfactant secretion and Na\(^{+}\) absorption) has not been reported. We adopted the following stepwise strategy to test our hypothesis: 1) identification of putative PGE\(_{2}\)-binding sites in the term fetal lung alveolar type II pneumocyte apical membrane by radioligand binding; 2) exploration of the PG effect on G protein function in this membrane (because PGs act via G proteins in other tissues; see Refs. 16 and 26); and, finally, 3) study of possible PGE\(_{2}\)-mediated regulation of Na\(^{+}\) transport in the same preparation. High-affinity GTPase activity was measured as a marker of G protein function; this method of measurement and its usefulness in assessing tissue G protein turnover have been established already (20). The measurement of the rate of Na\(^{+}\) uptake into vesicles of type II pneumocyte apical membrane has been validated by our group as a marker of conductive Na\(^{+}\) transport (8); this method was utilized to study the possible effect of PGE\(_{2}\) on Na\(^{+}\) channels in the type II pneumocyte apical membrane.
MATERIALS AND METHODS

Materials

[\gamma\-32P]GTP (specific activity 10 Ci/mmole; Amersham, Bucks, UK), [3H]PGE2 (specific activity 171 Ci/mmole; Du Pont de Nemours), and [22Na] (specific activity 15 Ci/mmole; Amersham) were the radioactive stocks purchased. Unlabeled PGE1, PGE2, PGD2, and PGF2\alpha were purchased from Cascade (Reading, Berkshire, UK). All other reagents were of Sigma (Poole, Dorset, UK) analytical grade.

Methods

Isolation of type II pneumocyte apical membrane. Apical membrane was prepared from late-gestation (<3 days pre-term) fetal guinea pig lungs as previously described (8). Briefly, lung lobes were excised at 2nd/3rd generation bronchi, placed in ice-cold homogenization medium (250 mM sucrose and 10 mM triethanolamine (TRA), pH 7.6 with HCl), containing 200 µg deoxyribonuclease I, 2.6 mg aprotinin A, 1.0 mg leupeptin, 1.0 mg pepstatin A, and 17.5 mg 4-(2-aminoethyl)benzenesulfonfluoride per 100 ml of medium), and homogenized in a Waring blender followed by a Polytron emulsifier (5 min; 4°C for both). After this, differential centrifugation and Mg2+ precipitation (in 75 mM NaCl, 50 mM MgCl2, and 10 mM HEPES, pH 7.4 with NaOH) were employed to yield a membrane fraction that was enriched of various contaminant membranes) were carried out as previously described. Markers for endoplasmic reticulum, mitochondria, and lysosomal membranes were not significantly enriched. Protein was estimated by the method of Bradford (3), with bovine serum albumin as the standard.

Identification of putative PGE2-binding sites. A [3H]PGE2 binding assay was performed to identify putative PGE2 receptors in the above preparation. The conditions of the assay have been described earlier (7). Briefly, membrane (50 µg protein) was incubated at 23°C with the indicated amounts of [3H]PGE2 (earlier experiments had established that optimum radiolabeled PG binding was achieved at room temperature). Incubations were performed in triplicate in buffer (250 mM mannitol and 10 mM TRA, pH 7.6 with HCl) and was stored at 4°C, and the supernatant was assayed for P i using the molybdate blue method (11). PG was added as appropriate which in turn drives tracer22Na uptake in the appropriate (vehicle) control. Data are presented as means ± SE (when the number of experiments totaled three
or more). Iterative fitting was performed on the entire data set, but, in some figures, only the means were plotted for clarity. Where relevant, differences between sets of experiments were studied by the Mann-Whitney test (2-tailed), with significance accepted at \( P \leq 0.05 \).

**RESULTS**

Identification of Putative \( \text{PGE}_2 \)-Binding Sites

Binding of 10 nM \(^3\text{H}\) \( \text{PGE}_2 \) was time dependent and saturable, with equilibrium being attained within 30 min of incubation, which was the time point chosen for all subsequent equilibrium binding experiments (data not shown). Addition of a large excess of unlabeled \( \text{PGE}_2 \) (1,000-fold) to the reaction mixture at the beginning of the incubation reduced the binding of \(^3\text{H}\) \( \text{PGE}_2 \) to the membrane to \( \sim 40\% \) of the total (see Fig. 2A), indicating the competitive nature of \(^3\text{H}\) \( \text{PGE}_2 \) binding. The binding isotherm shown in Fig. 1A is well fitted by a two-site model with affinities (\( K_D \)) of 2.11 ± 0.70 and 27.83 ± 1.99 nM and \( B_{max} \) of 27.46 ± 6.89 and 265.44 ± 29.13 fmol/mg protein. Separate theoretical isotherms using these calculated parameters are also shown for information. Transformation of the data to yield a Scatchard plot (Fig. 1B) clearly shows the presence of two binding sites. Incubation of the lung apical membrane with trypsin (53.5 U/50 µg protein in 200 µl buffer) completely abolished specific \(^3\text{H}\) \( \text{PGE}_2 \) binding (data not shown). The specificity of \(^3\text{H}\) \( \text{PGE}_2 \) binding was investigated by adding increasing concentrations (0.001–2.5 µM) of unlabeled PGs \( \text{E}_1, \text{E}_2, \text{D}_2, \) and \( \text{F}_2a \) to the reaction mixture. Fitting the dose-response data using the Hill equation (which was plotted on a linear concentration axis; Fig. 2, A–D) showed that \( \text{PGE}_2 \) and \( \text{PGE}_1 \) had similar affinities (Fig. 2, A and B) for the receptor (\( IC_{50} \) for \( \text{PGE}_2 \) self-inhibition = 47.4 ± 19.0 nM; \( IC_{50} \) for \( \text{PGE}_1 \) inhibition of \( \text{PGE}_2 \) binding = 35.4 ± 6.0 nM). However, \( \text{PGD}_2 \), although able to inhibit \( \text{PGE}_2 \) binding (Fig. 2C), did so with reduced affinity (\( IC_{50} = 412 ± 143 \) nM). \( \text{PGF}_2a \), clearly produced minimal inhibition (Fig. 2D), and \( IC_{50} \) values could not be reliably calculated from the data.

**PG Effect on \( \text{G Protein Function} \)**

The dose responses of \( \text{PGE}_1, \text{PGE}_2, \text{PGD}_2, \) and \( \text{PGF}_{2a} \) on high-affinity \( \text{G}_\alpha \)-GTPase activity was studied between 1 nM and 1 mM (Fig. 3). With the use of the Hill equation, calculated \( IC_{50} \) values for inhibition of GTPase activity by \( \text{PGE}_2 \) (29.3 ± 12.2 µM), \( \text{PGE}_1 \) (24.9 ± 14 µM), and \( \text{PGD}_2 \) (40.0 ± 17.2 µM) are more than two orders of magnitude larger than that of \( \text{PGF}_{2a} \) (11.3 ± 9.2 mM). The \( IC_{50} \) values for \( \text{PGE}_2 \) and \( \text{PGE}_1 \) are similar, whereas the \( IC_{50} \) for \( \text{PGD}_2 \) is greater than that for either \( \text{PGE}_2 \) or \( \text{PGE}_1 \) (although the difference did not achieve significance). The \( IC_{50} \) for \( \text{PGF}_{2a} \) is greatly different (\( P < 0.05 \)) from all of the other PGs. This rank order of potency for inhibition of \( \text{G}_\alpha \)-GTPase activity is in agreement with that obtained in the binding studies. The Hill coefficient of all the curves was significantly less than one (manifest as shallow log dose-response curves). This is compatible with two populations of \( \text{G protein-linked receptors, each exhibiting an affinity about one order of magnitude different from the other.} \)

**\( \text{PGE}_2 \)-Mediated Regulation of \( \text{Na}^+ \) Transport**

Figure 4 shows that extravesicular \( \text{PGE}_2 \) (1 µM) or intravesicular GTP (100 µM) in isolation produced no significant change in the initial rate of \(^2\text{Na}^+\) uptake (\( n = 7 \)). In combination, however, these concentrations...
of PGE$_2$ and GTP significantly increased the initial rate of $^{22}$Na$^+$ uptake 1.38 ± 0.09-fold above control ($P < 0.05; n = 7$), indicating that PGE$_2$ stimulation of apical Na$^+$ channels is G protein-dependent. The differences between the effect of PGE$_2$ and GTP in combination and that of PGE$_2$ or GTP alone were also significant ($P < 0.05; n = 7$). There was no significant difference between the increase in the initial rate of $^{22}$Na$^+$ uptake achieved with intravesicular GTP$_s$ (100 µM) and that achieved with PGE$_2$ and GTP in combination ($n = 7$), suggesting that PGE$_2$ could maximally activate the G protein-dependent component of Na$^+$ transport through conductive pathways.

**DISCUSSION**

This paper confirms the presence of a high-affinity PGE$_2$-binding site on the type II pneumocyte apical membrane of the term guinea pig fetus. Kinetic analysis of $[^3H]$PGE$_2$ binding to type II pneumocyte apical membrane shows that this binding is saturable and reversible. The ligand affinity is similar (i.e., within 1
Although PGE2 and PGE1 were approximately equally for its characterization. It has been shown that although PGE2 and PGE1 are roughly equipotent, whereas PGD2 and PGF2α, (in that order) show an apparently diminished effect on inhibition of G protein turnover. This match between rank order of ligand binding and G protein turnover inhibition is further suggestive of the presence of a functional PG receptor working via G proteins in the fetal alveolar type II cell apical membrane.

Because PGF2α, PGE1, PGD2, and PGF2α, have well-characterized Na⁺-absorptive roles in the kidney (26), we investigated their relative potencies in the lung alveolar apical membrane by studying their ability to displace [3H]PGE2 from the putative receptor site. Although PGE2 and PGE1 were approximately equally effective in competing with [3H]PGE2, PGD2 showed a diminished effect, followed by PGF2α. We identified a similar differential in the PG effect on high-affinity GTPase activity in the same preparation: PGE2 and PGE1 are roughly equipotent, whereas PGD2 and PGF2α, (in that order) show an apparently diminished effect on inhibition of G protein turnover. This match between rank order of ligand binding and G protein turnover inhibition is further suggestive of the presence of a functional PG receptor working via G proteins in the fetal alveolar type II cell apical membrane.

The ability of PGs to displace [3H]PGE2 has been recently utilized to classify EP receptors (receptors apparently specific for PGE2; see Ref. 5). Although not studied here, we have previously shown that ~70% of the basal high-affinity GTPase activity in this preparation is pertussis toxin insensitive. Interestingly, an EP receptor has been shown to be coupled via the pertussis toxin-insensitive G protein Gq (possibly one of the functionally important G proteins in our system; see Ref. 21) in bovine adrenal glands (22). Our observed rank order (PGE2 = PGE1 > PGD2 > PGF2α) for [3H]PGE2 displacement is characteristic of the EP3 subtype (5). Although EP3 receptor mRNA is expressed in many tissues, it is of relevance that expression is significant in both kidney and lung (31).

Finally, we studied the effect of stimulating this G protein-linked PG receptor on Na⁺ transport into apical membrane vesicles. It is appropriate at this point to mention the caveat that agonist-dependent stimulation of the initial rate of 22Na⁺ uptake into vesicles represents data obtained from a pure system and may not replicate events at the complex level of the cell and in the animal. The system allows us, however, to study more specifically the regulation of conductive Na⁺ transport in type I pneumocytes while generating data that we expect will be interpretable in due course in the context of a functionally integrated preparation.

In apical membrane vesicles, PGE2 (1 µM) or GTP (100 µM) on their own had no significant effect on the initial rate of 22Na⁺ uptake compared with control. In combination, however, they produced a 38% increase in this rate. Interestingly, this is not significantly different from the increase in initial rate achieved by irreversible G protein activation alone with the nonhydrolyzable GTP analog GTPγS. Because all PG receptors are G protein linked, it follows that stimulation of the alveolar prostanoïd receptor is capable of fully activating the G protein-mediated upregulation of lung alveolar Na⁺ transport. Thus (as for the renal Na⁺ absorptive process), PGE2 receptors may prove to be an important determinant of overall perinatal alveolar fluid homeostasis, facilitating the clearance of excess lung fluid in preparation for birth.

Although inhibition of G protein activity with PGs (23) and other agonists (9) has been previously observed, we are the first to note the apparent association of such inhibition with an upregulation of cellular function. G protein-mediated signal transduction is not a simple linear process but involves intricate cross talk between different G protein-coupled pathways and between G protein-coupled pathways and other signal transduction systems (29). Gβγ dimer could regulate this interaction between G protein and the Na⁺ channel, since maintaining the Gα-GTP state will hinder reassociation of the heterotrimeric G protein and could facilitate possible Gβγ-mediated stimulation of Na⁺ transport. Also, both stimulation and inhibition of G proteins regulate surfactant secretion in the type II pneumocyte (25), and further studies are necessary to relate the observations of downregulation of high-affinity GTPase activity and stimulation of Na⁺ channel function. We (21) and other workers (9) have recently observed similar G protein turnover inhibition induced by polyunsaturated fatty acids. Although others have discussed possible mechanisms by which such an effect on G proteins could be of functional importance by being transiently inhibitory (thus allowing participation in a feedback regulatory process), we have explored its relation to concomitant upregulation of cellular function.

Fig. 4. PGE₂ stimulates the initial rate of conductive ²²Na⁺ uptake into type II pneumocyte apical membrane vesicles. Apical membrane was vesiculated in the presence of Mg²⁺ (1 mM). Results are presented as proportional increase in the initial rate of ²²Na⁺ uptake compared with control (means ± SE; n = 7). As observed earlier (see text), intravesicular guanosine 5'-O-(3-thiotriphosphate) (GTPγS; 100 µM) consistently stimulated conductive ²²Na⁺ uptake by causing irreversible G protein activation (P < 0.05). Intravesicular GTP (100 µM) and/or extravesicular PGE₁ (1 µM) was added as indicated. GTP and PGE₂ alone had no significant effect on ²²Na⁺ uptake. In combination, they significantly increased ²²Na⁺ uptake. A significant difference is observed between the effects of 1) PGE₂ and GTP + PGE₂ and 2) GTP and GTP + PGE₂ (P < 0.05).
Neonatal respiratory distress syndrome continues to have a high mortality and morbidity despite the introduction of surfactant treatment (27). Novel therapeutic strategies directed toward lung alveolar fluid overload (a problem that coexists with surfactant depletion in respiratory distress syndrome) are likely to improve survival and reduce morbidity. A clear understanding of the cellular pathways that regulate Na+–driven absorption, like that described here of PG receptors, of lung fluid at birth is of crucial importance in the formulation of such strategies.

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