Evidence that PGE\textsubscript{2} Stimulates Intestinal Epithelial Cell Adenylate Cyclase by a Receptor-Mediated Mechanism

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These studies were performed to examine whether prostaglandin E\textsubscript{2} stimulates intestinal epithelial secretion via a receptor-mediated or non-receptor-mediated activation of adenylate cyclase. Solubilization of epithelial cell adenylate cyclase with LubroI PX, which separates the receptor moiety of the cyclase from the remainder of the complex, inhibited the prostaglandin E\textsubscript{2} stimulation of the cyclase. A similar result was obtained with VIP, which activates adenylate cyclase via a receptor-mediated mechanism, whereas fluoride, γ\textsubscript{S}-GTP, and forskolin, which activate the cyclase via non-receptor-mediated mechanisms, all stimulated solubilized adenylate cyclase. In addition, prostaglandin E\textsubscript{2} and VIP both showed a dependence on GTP for adenylate cyclase stimulation while fluoride and forskolin did not. These data suggest that prostaglandin E\textsubscript{2} activates intestinal mucosal adenylate cyclase by a receptor-mediated mechanism. The presence of such receptors lends support to the possibility that prostaglandins have a physiological role in the control of mucosal transport.

Prostaglandins can induce small intestinal secretion (1) and, since they are synthesized locally in the gut, they may have a role in the control of secretion (2, 3). We have previously demonstrated that a major site of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) synthesis is in the subepithelial layer of rat small intestine (4). Degradation, on the other hand, occurs predominantly in the epithelium (4), suggesting the possibility that prostaglandins, after being synthesized subepithelially, may regulate ion and water transport by exerting their action on the epithelium where they are subsequently degraded. Prostaglandins have been shown to elevate cAMP levels in vivo (5) by stimulation of an adenylate cyclase on the basolateral membrane of epithelial cells (6, 7). According to this scheme, the activation of adenylate cyclase might be expected to be receptor mediated but the existence of receptors for PGE\textsubscript{2} has not yet been demonstrated. In this paper we describe investigations into the possible involvement of receptors for PGE\textsubscript{2} in the activation of intestinal epithelial plasma membrane adenylate cyclase.

MATERIALS AND METHODS

Epithelial Cells. Cells were prepared by the Ca\textsuperscript{2+} chelation/vibration method described previously (4). After collection, the cells were either washed three times with cell suspension buffer containing IBMX (sucrose, 200 mM; disodium hydrogen phosphate, 76 mM; potassium dihydrogen phosphate, 19 mM; IBMX, 0.5 mM; pH 7.4) and finally resuspended in this buffer to a protein concentration of approximately 10 mg/ml ready for use,
or used as the starting material for the preparation of plasma membranes.

**Plasma Membranes.** A semipurified plasma membrane fraction was prepared from isolated epithelial cells as described by Murer et al (7). Briefly, the epithelial cells from two rats were homogenized in 40 ml buffer containing sucrose, 250 mM; triethanolamine, 10 mM; EDTA, 0.5 mM, pH 7.5, with 10 strokes of a Dounce homogenizer. After successive centrifugations at 2600g (15 min) and 10,000g (20 min), the pelleted membranes were washed by recentrifugation in the homogenization buffer (2 x 20,000g; 20 min). The final pellet was resuspended in a buffer containing sucrose (250 mM) and HEPES (10 mM) to a final protein concentration of approximately 2 mg/ml and stored at -70 °C until required. The semipurified membranes were enriched in both brush-border and basolateral enzyme markers but were not significantly contaminated with microsomal or mitochondrial components.

**Solubilization.** We took advantage of the observation that receptor-mediated activation of adenylate cyclase is lost if the adenylate cyclase is liberated from the membrane (and the receptor part of the complex) by the detergent Lubrol PX. Non-receptor-mediated activation, as, for instance, by fluoride, is unaffected by solubilization.

Membranes were solubilized by mixing with a buffer containing Lubrol PX to give a final concentration of: sucrose 250 mM, Tris HCl, 25 mM; EDTA, 1 mM; MgCl₂, 2 mM; and Lubrol at 0, 0.1, 0.2, 0.5, or 1%. After mixing and being allowed to stand for 15 min on ice, the suspensions were centrifuged at 100,000g for 60 min. The supernatants were then carefully removed and used as the Lubrol-soluble fraction. The pellets were resuspended in the same volume of an equivalent buffer with Lubrol. These fractions were used as the Lubrol-insoluble fractions.

**Adenylate Cyclase Activity.** In whole cells the activity was determined by addition of 450 μl of cell suspension, in sucrose phosphate buffer containing IBMX, to 50 μl of saline containing the required amount of PGE₂. After mixing by inversion, the cells were incubated for 3 min at 30°C. The assay was stopped by boiling for 3 min followed by centrifugation to sediment the denatured protein.

In isolated membranes, and Lubrol-treated fractions, adenylate cyclase was measured as described previously (4) except that in some experiments the influence of varying concentrations of GTP was assessed. Assays were performed for 10 min at 30°C and stopped by boiling for 3 min followed by centrifugation to sediment the denatured protein. The cAMP produced was determined by a competitive binding assay.

Protein was determined by the method of Lowry et al (8) with bovine serum albumin as standard. Forskolin was obtained from Calbiochem-Behring Corp and γS-GTP, guanosine S′-O(3-thiotriphosphate) from Boehringer Corporation (London) Ltd. All other biochemicals were obtained from the Sigma Chemical Company, Ltd., London.

**RESULTS**

**PGE₂ Dose Response.** PGE₂ stimulated a dose-dependent increase in epithelial cell cAMP levels (Figure 1A) and in adenylate cyclase activity measured directly in a plasma membrane fraction isolated from these cells (Figure 1B). A 4.5-fold increase over basal cyclase activity occurred in the plasma membrane fractions with 10⁻⁴ M PGE₂, while only a 1.6-fold stimulation in cAMP levels was found in the whole cell population. Cellular cAMP levels are a balance between synthesis (cyclase activity) and degradation (phosphodiesterase activity) and are not directly equivalent to cyclase activities, but it is interesting that the ED₅₀ of the PGE₂ response is 10⁻⁶ M in both cases.

**Solubilization.** Solubilization by Lubrol caused a significant release of cyclase activity from the membrane fraction. This was virtually maximal at 0.1% Lubrol and accounted for >90% of the total forskolin-stimulated activity associated with the membranes (a similar pattern was produced for fluoride and γS-GTP stimulated activity). The disappearance of activity from the particulate fraction was accompanied by an increase in activity appear-

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