A Prostaglandin E Receptor Coupled to a Pertussis Toxin-sensitive Guanine Nucleotide Regulatory Protein in Rabbit Cortical Collecting Tubule Cells*

(Received for publication, November 2, 1989)

William K. Sonnenburg, Jianhua Zhu, and William L. Smith†

From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

At different concentrations, prostaglandin E₂ (PGE₂) can either stimulate or inhibit cAMP formation in freshly isolated rabbit cortical collecting tubule (RCCT) cells, but in cultured RCCT cells PGE₂ can only stimulate cAMP synthesis (Sonnenburg, W. K., and Smith W. L. (1989) J. Biol. Chem. 264, 6155-6160). Here, we report characteristics of [³H]PGE₂ binding to membrane receptor preparations from both freshly isolated and cultured RCCT cells. [³H]PGE₂ binds to membranes from freshly isolated RCCT cells was saturable and partially reversible. Equilibrium binding analyses indicated that in the absence of guanosine 5'-3-O-(thio)triphosphate (GTPγS) there is a single class of PGE₂ binding sites (K_d = 4.2 ± 0.4 nM; B_max = 583 ± 28 fmol/mg); in the presence of 100 μM GTPγS, there is also only one class of binding sites but with a somewhat lower K_d = 1.2 ± 0.5 nM (B_max = 370 ± 40 fmol/mg). This stimulatory effect of GTPγS was blocked by pretreatment of the freshly isolated RCCT cells with pertussis toxin. The relative affinities of prostaglandins for the [³H]PGE₂ binding site were determined to be 17,18,19,20-tetranor-16-phenoxy-PGE₂ > methylsulfonylamide (prostacyclin) > PGE₂ ≈ PGE₁ ≈ 16,16-dimethyl-PGE₂ > carbacyclin ≈ PGF₂α > PGE₁. This is the order of potency with which prostaglandins inhibit arginine vasopressin-induced cAMP formation in fresh RCCT cells. Interestingly, [³H]PGE₂ binding to membranes from cultured cells, which, unlike fresh cells, fail to show an inhibitory response to PGE₂, was only 10-20% of that observed with membranes from fresh cells: moreover, binding of [³H]PGE₂ to membranes from cultured cells was neither stimulated by GTPγS nor inhibited by sulprostone. The prostaglandin binding specificities and the unusual pertussis toxin-sensitive, stimulatory effect of GTPγS on binding of [³H]PGE₂ to membranes from freshly isolated RCCT cells are characteristics shared by a G-i linked PGE receptor from renal medulla (Watanabe, T., Umegaki, K., and Smith, W. L. (1986) J. Biol. Chem. 261, 14340-14349). Our results suggest that the [³H]PGE₂ binding site of freshly isolated RCCT cells is the PGE receptor which is coupled to a G_i to attenuate arginine vasopressin-induced cAMP synthesis in the renal collecting tubule.

In a previous report (1), we established that prostaglandin E_2 derivatives, including the synthetic analog, sulprostone, act at low concentrations (about 10^{-9}-10^{-10} M) to inhibit arginine vasopressin (AVP)-induced adenylate cyclase activity in freshly isolated rabbit cortical collecting tubule (RCCT) cells. This inhibitory effect, which was blocked by pertussis toxin, was not detectable in RCCT cells which had been grown in primary culture, although an α_2-adrenergic, G_i-linked response was detectable in the cultured cells. We also found that PGE_2 derivatives, with the notable exception of sulprostone, act at somewhat higher concentrations (≥10^{-7} M) to stimulate cAMP synthesis and that a stimulatory response to PGE_2 was observed not only with freshly isolated RCCT cells, but also with cultured cells.

These results suggested that PGE_2 exerts its effects on cAMP metabolism in the rabbit cortical collecting tubule by interacting with two distinct PGE receptors: (a) a high affinity, pertussis toxin-sensitive inhibitory PGE receptor which binds both PGE_2 and sulprostone and is present only in freshly isolated RCCT cells, and (b) a lower affinity, pertussisin-sensitive stimulatory PGE receptor which interacts with PGE_2, but not sulprostone, and is present in both freshly isolated and cultured RCCT cells. To begin testing this model, we have characterized [³H]PGE₂ binding to membranes prepared from both freshly isolated and cultured RCCT cells. Reported here are the results of these studies, as well as the results of related studies designed to determine if sulprostone is equipotent to PGE₂ in interacting with the G_i-linked PGE receptor of renal outer medulla (2).

EXPERIMENTAL PROCEDURES

Materials—Reagents were obtained from the following sources: [5,6,8,11,12,14,15(H)-³H]PGE₂ (160-200 Ci/mmol) from Du Pont-New England Nuclear; collagenase, RPMI 1640, and fetal bovine serum from Grand Island Biological Co.; PGF₂α, PGE₂, PGF₁α, and 16,16-dimethyl-PGE₂ from Cayman Chemical Co.; PGD₂ from Upjohn Diagnostics; flurbiprofen and carbacyclin were gifts from Dr. John Pike of the Upjohn Co.; transferrin and guanosine 5'-3-O-(thio)triphosphate (GTPγS) from Boehringer Mannheim; GF/F glass microfiber filters (25 mm) from Whatman; Safety-Solve scintillation fluid from Research Products Int.; pertussis toxin from List Biological Laboratories; sulprostone was a gift from Berlex Laboratories, Inc.

Isolation and Culture of RCCT Cells—RCCT cells were isolated by immersion adsorption as described previously (3) using polylysine culture dishes coated with a monoclonal antibody that reacts with an

* This work was supported by National Institutes of Health Grants DK22942 and DK36485, a grant-in-aid from the American Heart Association of Michigan, and National Institutes of Health Postdoctoral Training Grant HL-07404. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed.

‡ The abbreviations and trivial names used are: sulprostone, 16-phenoxo-17,18,19,20-tetranor-PGE-methylsulfonylamide; AVP, arginine vasopressin; PGE₂, PGE₁, PGF₂α, PGD₂, and PGI₂, prostaglandins E₂, E₁, F₂α, D₁, and I₂; carbacyclin, 6-carbaprostaglandin I₂; RO 20-1724, (±)-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; GTPγS, guanosine 5'-3-O-(thio)triphosphate; HEPES, 4-(2-hydroxyethyle)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxy-ethylenenitrilo)]tetraacetic acid.
ectoantigen specific for the collecting tubule. Cells to be cultured were initially grown in RPMI 1640 medium supplemented with transferrin (5 μg/ml) and 3% fetal bovine serum until confluent (3-5 days). RCCT cells were subsequently cultured for 48 h in the same medium without serum (4). All incubations were carried out in a 37 °C incubator with a water-saturated 7% CO2/air atmosphere. Each isolation (6 rabbit kidneys) routinely yielded enough freshly isolated cells to prepare 4-6 mg of membrane protein.

Preparation of Membranes from RCCT Cells—All buffers used to prepare membranes contained the cyclooxygenase inhibitor, flurbiprofen. Prior to preparing membranes, cultured cells were pretreated for 4 h with flurbiprofen, changing the medium once after the first 30 min. RCCT cells (either freshly isolated or cultured) were scraped from tissue culture plates using a rubber policeman, resuspended in 10 mM HEPES, pH 7.5, and centrifuged for 15 min at 30,000 x g. The resulting membranes were washed twice by resuspending the pellet in 10 mM HEPES, pH 7.5, containing 2 mM EDTA using a Dounce homogenizer and centrifuged as described above. The final membrane pellet was resuspended in HEPES, pH 7.5, containing 1 mM MgCl2 using a Dounce homogenizer, yielding a final protein concentration of 1.5 mg/ml. Membrane protein was determined by a modification (5) of the Lowry method.

Preparation of Membranes from Canine and Rabbit Renal Outer Medulla—Canine kidneys were obtained immediately after killing by CO2 from the Clinton County (MI) Animal Control Department. Rabbit kidneys were obtained from 2.5-kg male New Zealand White rabbits killed by injection of 5 ml of 5% Nembutal via the marginal ear vein. Typically, the outer (red) medulla was dissected and stored at −80 °C before use of the tissue. The tissue was thawed and membranes prepared by a modification of procedures described previously (2). Typically, 2.5 g of tissue was homogenized at 4 °C in 25 ml of 10 mM HEPES, pH 7.5, containing 2 mM EDTA, 0.25 M sucrose, 2 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, and 40 μM indomethacin with three 15-s bursts at top speed on a Polytron homogenizer. The sample was centrifuged at 10,000 × g for 10 min and the resulting supernatant filtered through eight layers of cheesecloth and centrifuged at 35,000 × g for 30 min. The resulting pellet was resuspended in 10 ml of 10 mM HEPES, pH 7.5, containing 2 mM EDTA, 0.4 μM sucrose, 2 mM indomethacin and three 15-s bursts at top speed on a Polytron homogenizer. The sample was centrifuged at 10,000 × g for 10 min and the resulting supernatant filtered through eight layers of cheesecloth and centrifuged at 35,000 × g for 30 min. This washing procedure was again repeated and the final membrane pellet resuspended in 10 mM HEPES, pH 7.5, containing 1 mM MgCl2 and 40 μM indomethacin at a concentration of 2-4 mg of protein/ml. Membrane preparation contained on the average 70% of the specific [3H]PGEl binding activity present in whole homogenates.

PGEl Binding Assays—Total [3H]PGE binding was determined essentially as described previously (2). RCCT cell or canine or rabbit outer medullary membrane (60-180 μg of protein/ml) were incubated at 30 °C in a reaction mixture containing the following components: 50 mM Tris maleate, final pH 5.5, 1 mM MgCl2, and 2 nM [3H]PGEl in a final volume of 200 μl. In some experiments, other additions were made and are noted in the text. Nonspecific binding was determined in parallel by incubating membranes under the same conditions but in the presence of 10 μM unlabeled PGE. The binding assays were stopped by adding 4 ml of ice-cold 50 mM Tris maleate, pH 5.5, and rapidly filtering the sample through a Whatman GF/F filter. The filter was immediately washed three times with 4 ml of 50 mM Tris maleate, pH 5.5. Each filter was placed into a scintillation vial containing 10 ml of Safety-Solve and bound radioactivity was quantitated by liquid scintillation counting.

As would be expected from the results of the equilibrium binding analyses shown in Figs. 2 and 3, GTPγS stimulated

FIG. 1. Time course of [3H]PGE2 binding to membranes prepared from freshly isolated RCCT cells. Membranes were prepared as described in the text and incubated with 2 nM [3H]PGE2 at 30 °C for the indicated times (O). In addition, 10 μM PGE2 was added to samples preincubated for 1 h, and [3H]PGE2 binding to these samples was measured (O). Binding assays were performed in duplicate.

The values for nonspecific binding were subtracted from the total binding values to yield specific PGE2 binding. Nonspecific binding averaged 15% (range: 10-20%) for membranes from freshly isolated cells, and 50% (range: 45-55%) for membranes from cultured RCCT cells. All values are expressed as specific binding except as noted in the text. For determination of Kd values and for competitive displacement studies, varying concentrations of unlabeled ligand were added to the reaction mixture. The binding isotherms were analyzed by computerized nonlinear curve fitting using the LIGAND program (6).

Pertussis Toxin Treatments—Freshly isolated or cultured RCCT cells were pretreated with or without pertussis toxin (1 μg/ml, final) in RPMI 1640 medium for 4 h at 37 °C. The cells were washed with RPMI medium and scraped from the culture dish and membrane prepared as described above.

Statistical Analyses—In some experiments, differences among treatment groups were determined using a completely random analysis of variance. Treatment means were compared using the least significance difference test or Student-Newman-Keul's test, where applicable (p ≤ 0.05) (7).

RESULTS

[3H]PGE2 Binding to Membranes Prepared from Freshly Isolated Rabbit Cortical Collecting Tubule Cells—Specific binding of 2 nM [3H]PGE2 to membranes prepared from freshly isolated RCCT cells was saturable and reached equilibrium in about 1 h at 30 °C. In the experiment depicted in Fig. 1, nonspecific binding averaged 15 ± 5% of total binding. In the experiment depicted in Fig. 1, bound [3H]PGE2 was only partially displaced (55%) upon addition of an excess of unlabeled PGE. In four separate experiments, displacement ranged from 55 to 86%. To determine if the bound [3H]PGE2 had been catabolized or had become covalently linked to the membranes, we incubated membranes for 2 h with 2 nM PGE2, collected the membranes by filtration, and extracted the washed membranes with CHCl3:methanol (2/1, v/v). Greater than 90% of the bound radioactivity was found to be lipid soluble and greater than 95% of this radioactivity co-chromatographed with authentic PGE2 in a thin layer chromatography system which separates PGE2 from potential polar (PGF2), and nonpolar (15-keto-PGE2) catabolites (8). Thus, the reason for the lack of complete displacement of [3H]PGE2 by excess unlabeled PGE2 is unclear, although it has been observed previously with [3H]PGE2 binding to canine renal medulla (2) and to rat adipocyte membranes.

Shown in Fig. 2 is a Scatchard plot of equilibrium binding of [3H]PGE2 to membranes prepared from freshly isolated RCCT cells. Data analyses by computerized nonlinear curve fitting (6) indicate that the best fit for the data is with a single site model with Kd = 4.2 ± 0.5 nM and Bmax = 583 ± 28 fmol/mg protein. Scatchard analysis of [3H]PGE2 binding to RCCT cell membranes in the presence of 100 μM GTPγS (Fig. 3) also revealed a single class of PGE2 binding sites, but with a significantly lower dissociation constant (Kd = 1.2 ± 0.5 nM; Bmax = 370 ± 40 fmol/mg protein). This observation was confirmed in two additional experiments, where GTPγS was found to reduce the apparent Kd values for [3H]PGE2 binding to membranes from freshly isolated RCCT cells from 8.6 to 2.8 and from 14.5 to 3.5 nM, respectively.

4G. Rimon, personal communication.
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Thus, the stimulatory effect of GTPγS on \(^{3}H\)PGE\(_2\) binding appears to involve a pertussis toxin-sensitive guanine nucleotide regulatory protein.

A concentration of 2 nM \(^{3}H\)PGE\(_2\) was also used in assays to determine the abilities of various prostanoids to inhibit \(^{3}H\)PGE\(_2\) binding to membranes from freshly isolated RCCCT cells. As shown in Figs. 5 and 6 and summarized in Table I, the E-series prostaglandins PGE\(_2\), PGE\(_3\), sulprostone, and 16,16-dimethyl-PGE\(_2\) were about equally effective in compet-

![Graph](image)

**Fig. 2.** Scatchard analysis of total \(^{3}H\)PGE\(_2\) binding to membranes from freshly isolated RCCCT cells. Binding assays were performed in duplicate at 30 °C for 2 h as described in the text in the presence of varying concentrations of PGE\(_2\). The solid line is a computer-generated regression (6) line for the binding site.

![Graph](image)

**Fig. 3.** Scatchard analysis of total \(^{3}H\)PGE\(_2\) binding to membranes from freshly isolated RCCCT cells in the presence of 100 μM GTPγS. Binding assays were performed in duplicate at 30 °C for 2 h as described in the text in the presence of varying concentrations of PGE\(_2\). The solid line is a computer-generated regression line (6) for the PGE-binding site.

![Graph](image)

**Fig. 4.** The effect of GTPγS on specific PGE\(_2\) binding to membranes from freshly isolated RCCCT cells pretreated with or without pertussis toxin. Freshly isolated RCCCT cells were pretreated for 4 h at 37 °C with vehicle or pertussis toxin as described in the text. Membranes were prepared and binding assays were performed in the presence (hatched bars) or absence (open bars) of 100 μM GTPγS for 2 h at 30 °C as described in the text. Values represent the mean of triplicate determinations ± S.E. Asterisk, significantly different than control values (p < 0.05).

![Graph](image)

**Fig. 5.** Inhibition of specific \(^{3}H\)PGE\(_2\) binding by various prostanoids in membranes from freshly isolated RCCCT cells. Binding assays were performed in duplicate at 30 °C for 2 h as described in the text in the presence of the indicated concentrations of PGE\(_2\) (○), sulprostone, PGE\(_3\), or 16,16-dimethyl-PGE\(_2\) (DM-PGE\(_2\)).

![Graph](image)

**Fig. 6.** Inhibition of specific \(^{3}H\)PGE\(_2\) binding by various prostanoids in membranes from freshly isolated RCCCT cells. Binding assays were performed at 30 °C for 2 h in duplicate as described in the text in the presence of the indicated concentrations of PGE\(_2\) (○), carbacyclin (○), PGF\(_{2\alpha}\) (A), or PGD\(_2\) (Δ).

Table I

| Prostanoid       | Fresh RCCCT cells | Rabbit medulla | Canine medulla |
|------------------|--------------------|----------------|----------------|
| PGE\(_2\)        | 9                  | 5.3            | 10             |
| PGE\(_3\)        | 8                  | 8.0            | 9              |
| Sulprostone      | 12                 | 15             | 6              |
| Carbacyclin      | 54                 | 49             | ND*            |
| PGF\(_{2\alpha}\)| 72                 | 100            | 80             |
| PGD\(_2\)        | 983                | 294            | 1000           |

*a ND, not determined.
pretreatment of cultured RCCT cells did not affect the ability of hormone-induced cAMP formation by freshly isolated and cultured RCCT cells which have been grown in primary culture. However, PGEz (but not sulprostone) stimulates cAMP formation by both freshly isolated and cultured RCCT cells (1). Some properties of [3H]PGEz binding to membranes from freshly isolated and cultured RCCT cells are compared in Fig. 7. Specific binding of 2 nM [3H]PGEz to membranes prepared from RCCT cells which had been grown in culture for 5-7 days was only 10-20% of that seen with membranes from freshly isolated cells and was near the limit of our detection; in fact, nonspecific binding of [3H]PGEz to membranes from cultured RCCT cells averaged about 50% of total binding (versus 15% with membranes from freshly isolated RCCT cells). Moreover, although GTPγS stimulated [3H]PGEz binding to membranes from freshly isolated cells, GTPγS actually inhibited binding of [3H]PGEz to membranes from cultured cells by about 65% (Fig. 7). Pertussis toxin pretreatment of cultured RCCT cells did not affect the ability of GTPγS to inhibit [3H]PGEz binding to membranes prepared from these cells (data not shown). Although sulprostone (10 μM) completely inhibited [3H]PGEz binding to membranes from freshly isolated RCCT cells, sulprostone had no significant effect on [3H]PGEz binding to membranes from cultured cells (Fig. 7).

[3H]PGEz Binding to Membranes from Rabbit and Canine Outer Medulla—We previously described the characteristics of a PGE receptor from canine renal outer medulla (2). This latter receptor could be solubilized in a complex with a Gt and GTP derivatives were found to stimulate [3H]PGE2 binding by decreasing the apparent Kd (2). As shown in Fig. 8A, sulprostone was as effective as PGEz in inhibiting the binding of [3H]PGEz to membranes from canine renal outer medulla. A PGE receptor with properties very similar to the receptor present in membranes from canine renal outer medulla was also found to be present in membranes prepared from rabbit outer medulla. The Kd value for [3H]PGEz binding to the rabbit outer medullary receptor was determined by Scatchard analysis to be 2 nM; moreover, GTPγS routinely stimulated binding 1.5- to 3.0-fold (data not shown). Sulprostone and PGEz were comparably effective in inhibiting the binding of [3H]PGEz to preparations of rabbit outer medullary membranes (Fig. 8B). Table I compares the abilities of a series of prostanoids to compete with [3H]PGEz for binding to membranes from freshly isolated RCCT cells and the GTP-stimulatable PGE receptors present in canine and rabbit outer medulla membranes. In all cases, PGEl and PGEz and sulprostone were comparably effective, while PGF2α was about 10-fold less effective, and PAGDz 30- to 100-fold less effective. Thus, the [3H]PGEz binding activities of membranes from freshly isolated RCCT cells and the GTP-stimulatable PGE receptors present in canine and rabbit outer medulla membranes all exhibit similar behavior with respect both to their binding specificities and to their unusual behavior upon addition of GTPγS.

**DISCUSSION**

Prostanoids of the E-series acting at concentrations of about 10-8 M inhibit AVP-induced water reabsorption in the rabbit cortical collecting tubule while at higher concentrations (>100 nM), PGEl and PGEz by themselves can stimulate water reabsorption (9, 10). As described in an earlier report (1), these responses can be rationalized on the basis of changes in cellular cAMP levels. E-series prostaglandins acting at low concentrations (0.1-10 nM) function via a pertussis toxin-sensitive mechanism to inhibit AVP-induced adenylate cyclase in freshly isolated RCCT cells and at higher concentrations PGEz acting alone can stimulate cAMP synthesis (1). In work described in this article, we have correlated prostanoid binding to RCCT cell membranes with the effects of prostanoids on cAMP metabolism in freshly isolated and cultured RCCT cells.
Our results indicate that there is a single, detectable \[^{[H]}\text{PGE}	ext{2}\] binding activity associated with membranes from freshly isolated RCCT cells. \(K_D\) values determined for \[^{[H]}\text{PGE}	ext{2}\] binding to this site in the presence of GTP\(\gamma\)S were in the range of 1–5 nM, consistent with a concentration of 3 nM PGE\(_2\) being able to cause half-maximal inhibition of AVP-induced cAMP formation in freshly isolated RCCT cells (1). The \[^{[H]}\text{PGE}	ext{2}\] binding activity of membranes from freshly isolated RCCT cells has a collage of properties expected for a G\(_i\)-linked PGE receptor which is coupled to inhibition of AVP-induced cAMP formation in the rabbit renal cortical collecting tubule. First, the order of potency for inhibition of AVP-induced cAMP formation by various prostanoids (1) is the same as the order of potency with which these same prostanoids inhibit the binding of \[^{[H]}\text{PGE}	ext{2}\] (i.e. sulprostone \(\approx\) PGF\(_2\alpha\) \(\approx\) PGE\(_2\) \(>\) 16,16-dimethyl-PGE\(_2\) \(>\) carbacyclin \(=\) PGF\(_{2\alpha}\) \(>\) PGD\(_2\) ); moreover, this rank order of potency differs from that seen for prostanoid-induced CAMP synthesis by RCCT cells (i.e. PGE\(_2\) \(>\) PGE\(_1\) \(>\) 16,16-dimethyl-PGE\(_2\) with sulprostone, PGF\(_{2\alpha}\), carbacyclin, and PGD\(_2\) being inactive (1)). Second, treatment of freshly isolated RCCT cells with pertussis toxin both blocks the ability of sulprostone and PGE to inhibit AVP-induced cAMP formation (1) and eliminates the GTP\(\gamma\)S effect on \[^{[H]}\text{PGE}	ext{2}\] binding to RCCT cell membranes. Third, the \[^{[H]}\text{PGE}	ext{2}\] binding activity of membranes from freshly isolated RCCT cells and the PGE receptor from canine renal medullary receptor, which has previously been solubilized in a complex with a G\(_i\), have very similar properties: (a) GTP\(\gamma\)S stimulates \[^{[H]}\text{PGE}	ext{2}\] binding, (b) the stimulatory effect of GTP\(\gamma\)S is blocked by pertussis toxin, and (c) the order of affinities for prostanoids are the same. And fourth, cultured RCCT cells, unlike fresh cells, lack an inhibitory G\(_i\) linked response to PGE\(_2\) and sulprostone (1), and cultured RCCT cells, unlike fresh cells, lack a pertussis toxin-sensitive sulprostone-inhibitable \[^{[H]}\text{PGE}	ext{2}\] binding activity. Based on these observations, we conclude that the \[^{[H]}\text{PGE}	ext{2}\] binding activity of freshly isolated RCCT cells represents the G\(_i\)-linked PGE receptor which mediates inhibition of AVP-induced cAMP synthesis in the renal cortical collecting tubule.

The effect of GTP\(\gamma\)S on \[^{[H]}\text{PGE}	ext{2}\] binding to membranes from freshly isolated RCCT cells is unusual and warrants a comment. Although guanine di- and trinucleotides typically decrease the affinity of G protein-linked receptors for their corresponding agonists in vitro (11, 12), GTP\(\gamma\)S actually stimulates binding of \[^{[H]}\text{PGE}	ext{2}\] to membranes from freshly isolated RCCT cells by decreasing the \(K_D\) value. However, it is unlikely that this effect of GTP derivatives has much significance in terms of regulating receptor response. It is sometimes assumed for intact cells (a) that receptors normally cycle between different affinity states depending on whether or not the G protein is occupied by a guanine nucleotide and (b) that this cycling has some kind of regulatory significance in vivo. However, binding of muscarinic agonists to intact cells appears to occur via a single affinity site with a \(K_D\) corresponding to that expected for a receptor interacting with a G protein occupied with a guanine nucleotide (13). Thus, \(K_D\) values measured in the presence of guanine nucleotides probably best reflect the \(K_D\) values for receptors of intact cells.

Our model for the actions of PGE\(_2\) in the collecting tubule indicates that there are both high and low affinity PGE receptors coupled to inhibition and activation of adenylyl cyclase, respectively (1). We have found no compelling evidence for the presence of a \[^{[H]}\text{PGE}	ext{2}\] binding activity other than the G\(_i\)-linked PGE receptor of freshly isolated RCCT cells. There is, however, a GTP\(\gamma\)S-inhibitable pertussis toxin-sensitive \[^{[H]}\text{PGE}	ext{2}\] binding activity associated with membranes from cultured RCCT cells. This binding activity could be distinguished from nonspecific binding, but the level of binding was too low to permit us to determine binding constants accurately. The fact that sulprostone fails to inhibit \[^{[H]}\text{PGE}	ext{2}\] to membranes from cultured RCCT cells raises the possibility that this binding activity corresponds to a PGE receptor coupled via G\(_i\) to the activation of adenylyl cyclase (14).

Acknowledgments—Flurbiprofen and carbacyclin were gifts from Dr. John Pike of the Upjohn Co. Sulprostone was a gift from Berlex Laboratories. RO 20-1724 was kindly provided by Dr. Peter Sorter of Hoffman-La Roche.

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J. Biol. Chem. 1990, 265:8479-8483.

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