Activation of FP Prostanoid Receptor Isoforms Leads to Rho-mediated Changes in Cell Morphology and in the Cell Cytoskeleton*

Kristen L. Pierce‡§, Hiromichi Fujino‡, Dinesh Srinivasan‡ and John W. Regan‡¶**

*This work was supported in part by National Institutes of Health Grant EY11291 and by a grant from Allergan, Inc. (to J. W. R.). 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.

§ Supported by an individual predoctoral fellowship from the National Science Foundation. Current Address: Box 3821, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.

** To whom correspondence should be addressed: Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona 85721. Tel.: 520-626-2181; Fax: 520-626-2466; E-mail: Regan@pharmacy.arizona.edu.

The abbreviations used are: PGF, prostaglandin F; PKC, protein kinase C; PMA, phorbol myristate acetate; EBNA, Epstein-Barr nuclear antigen; PIPES, 1,4-piperazinediethanesulfonic acid; TBS, Tris-buffered saline; TBS-T, TBS containing 0.1% (v/v) Tween 20; FAK, focal adhesion kinase.

Prostaglandin F$_2\alpha$ (PGF$_{2\alpha}$) exerts its biological effects by binding to and activating FP prostanoid receptors. These receptors, which include two isoforms, the FP$_A$ and FP$_B$, have been cloned from a number of species and are members of the superfamily of G-protein-coupled receptors. Previous studies have shown that the activation of FP receptors leads to phosphatidylinositol hydrolysis, intracellular calcium release, and activation of protein kinase C. Here, we demonstrate that PGF$_{2\alpha}$ treatment of 293-EBNA (Epstein-Barr nuclear antigen) cells that have been stably transfected with either the FP$_A$ or FP$_B$ receptor isoforms leads to changes in cell morphology and in the cell cytoskeleton. Specifically, cells treated with PGF$_{2\alpha}$ show retraction of filopodia and become rounded, and actin stress fibers are formed. Pretreatment of the cells with bisindolylmaleimide I, a protein kinase C inhibitor, has no effect on the PGF$_{2\alpha}$-induced changes in cell morphology, although it does block the effects of phorbol myristate acetate on cell morphology. On the other hand, the PGF$_{2\alpha}$-induced changes in cell morphology and formation of actin stress fibers can be blocked by pretreatment of the cells with C3 exoenzyme, a specific inhibitor of the small G-protein, Rho. Consistent with FP receptor induced formation of actin stress fibers and focal adhesions, FP$_A$ receptor activation also leads to rapid (within two minutes) tyrosine phosphorylation of p125 focal adhesion kinase (FAK) which can be blocked by pretreating the cells with C3 exoenzyme. Taken together, these results suggest that the FP receptor isoforms are coupled to at least two second messenger pathways, one pathway associated with protein kinase C activation, and the other with activation of Rho.

Prostaglandin F$_2\alpha$ (PGF$_{2\alpha}$)$^1$ is one of the biologically active prostanoids formed from the cyclooxygenase-catalyzed metabolism of arachidonic acid. Physiologically, PGF$_{2\alpha}$ is known to be important in regulating luteolysis or regression of the corpus luteum (1) and regulating intraocular pressure (2) and may also be involved in cardiac hypertrophy. Specifically, long term administration of the PGF$_{2\alpha}$ analog fluprostenol to rats led to an increase in both heart weight and ventricular weight relative to body weight (3). In vitro, PGF$_{2\alpha}$ treatment of isolated rat cardiomyocytes has been shown to lead to hypertrophy, to increased myofibrillar organization, and to increased atrial natriuretic factor expression (3–6).

PGF$_{2\alpha}$ has been shown to exert its physiological effects by binding to its receptor, the FP prostanoid receptor. This receptor is a member of the superfamily of G-protein-coupled receptors and has been cloned from a number of species, including human (7), mouse (8), bovine (9), and ovine (10). In addition, we recently cloned a carboxyl-terminal isoform of the ovine FP receptor, the FP$_B$ isoform (11). This truncated isoform, which arises by alternative mRNA splicing, lacks 46 amino acids present in the original isoform, now called the FP$_A$ receptor isoform. In heterologous expression systems, activation of either of the cloned FP receptor isoforms leads to an increase in inositol phosphate accumulation, protein kinase C (PKC) activation, and intracellular calcium release consistent with activation of G-proteins of the G$_q$ family (7–11).

Although the FP prostanoid receptor isoforms couple to activation of G$_q$, there is evidence that these isoforms may exert some of their physiological effects by activation of additional effectors. Strikingly, in isolated rat cardiomyocytes, the PGF$_{2\alpha}$-induced hypertrophy and atrial natriuretic factor expression is independent of PKC activation (5). This is surprising because PKC inhibitors do block the hypertrophic responses induced by other drugs, including phorbol myristate acetate (PMA) and phenylephrine (5). The insensitivity of the PGF$_{2\alpha}$-induced cardiac hypertrophy to PKC inhibitors suggests that PGF$_{2\alpha}$ may activate additional signal transduction pathways that are distinct from the known activation of G$_q$. Furthermore, activation of these pathways may be important in regulating some of the physiological actions of PGF$_{2\alpha}$, including cardiomyocyte hypertrophy.

To gain further understanding into the diversity of the signal transduction pathways activated by the FP$_A$ and FP$_B$ receptor isoforms, 293-EBNA cells that stably express each of these isoforms were generated, and the downstream signaling was examined. We now report that PGF$_{2\alpha}$ treatment of cells expressing either isoform leads to changes in cell morphology and in the cytoskeleton. These changes, which include retraction of filopodia, cell rounding, and the formation of actin stress fibers, are sensitive to the inhibition of the small G-protein Rho but not to the inhibition of PKC. These data suggest that the both the FP$_A$ and FP$_B$ receptor isoforms are coupled to at least two...
**Experimental Procedures**

*Stable Transfectants*—Stable transfectants of the FP<sub>A</sub> and FP<sub>B</sub> isoforms were generated in 293-EBNA cells (Invitrogen), a cell line derived from human embryonic kidney (HEK) cells that have been stably transfected with the Epstein-Barr nuclear antigen (EBNA). The presence of EBNA allows high copy episomal replication of plasmids, such as pCPE4 (Invitrogen) that contain an Epstein-Barr virus origin of replication. These cells were grown according to the manufacturer's instructions in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 250 μg/ml G418 (Life Technologies), and 200 μg/ml gentamicin (Life Technologies). For the stable transfection, the cDNA encoding the permeabilized for 15 min in 2× saline, quenched 3 times for 10 min in 0.1 M glycine, pH 7.4, and Nonidet P-40. Five rpm). Protein contents were normalized, and the maximum amount of The cell lysates were transferred to microcentrifuge tubes, and the samples were boiled and electrophoresed on 7.5% SDS-polyacrylamide gels. The electrophoresed proteins were transferred to nitrocellulose, and the blot was blocked overnight at 4 °C in 5 ml of 1× Tris-buffered saline (TBS, 10 mM Tris, pH 7.5, 100 mM NaCl) containing 0.1% (v/v) Tween 20 (Bio-Rad) (TBS-T) and 3% (w/v) bovine serum albumin. The blots were then incubated in this buffer with a 1:1,000 dilution of a monoclonal antiphosphotyrosine-specific antibody (PY-20, Transduction Labe) for 1 h at room temperature with rotation. The blots were washed several times in TBS-T and then incubated for 1 h at room temperature with a 1:5,000 dilution of a goat anti-mouse horseradish peroxidase-conjugated secondary antibody (Jackson Laboratories) in TBS-T plus 3% bovine serum albumin. After several washes in TBS-T, the immunoreactivity was detected using SuperSignal enhanced chemiluminescence (Pierce). To ensure equal loading of the blots, the blots were stripped and reprobed as before with a 1:1,000 dilution of the anti-FAK antibody (C-20, Santa Cruz Biotechnology) and a 1:10,000 dilution of a goat anti-mouse horseradish peroxidase-conjugated secondary antibody (Jackson Immunoresearch Laboratories, Inc.).

**C3 Exoenzyme Purification**—Escherichia coli transformed with a plasmid encoding a glutathione S-transferase/C3 exoenzyme fusion protein was used for the purification of the C3 exoenzyme according to Dillon and Feig (12). Fusion protein purification was performed using glutathione (GSH)-conjugated-Sepharose beads (Amersham Pharmacia Biotech), and thrombin (Haematological Technologies) was added to cleave the C3 exoenzyme from the glutathione S-transferase that remained bound to the GSH-Sepharose beads. The C3 exoenzyme that cleaved away from the bead was concentrated by centrifugation of approximately 4 μg/ml using a Centricon 10 (Amicon). The purity of the C3 exoenzyme was assessed by SDS-polyacrylamide gel electrophoresis.

**Results**

To examine the signal transduction pathways activated by the FP<sub>A</sub> and FP<sub>B</sub> receptor isoforms, the receptor isoforms were stably expressed in 293-EBNA cells. The FP<sub>A</sub> and FP<sub>B</sub> cell lines each express approximately 2 pmol of receptor/mg of total protein as assessed by radioligand binding using [3H]PGF<sub>2α</sub> (data not shown). We and others have previously demonstrated that the FP prostanoid receptor isoforms are coupled to the G-protein, G<i>q</i> (7–11), and as expected, PGF<sub>2α</sub> activation of the stably expressed FP<sub>A</sub> receptor and FP<sub>B</sub> isoforms led to an increase in inositol phosphate accumulation (EC<sub>50</sub> = 19 nM for the FP<sub>A</sub> and 11 nM for the FP<sub>B</sub>) and intracellular calcium release (data not shown).

Surprisingly, in addition to coupling to stimulation of intracellular calcium release and inositol phosphate production, we found that in cells that stably expressed either the FP<sub>A</sub> or FP<sub>B</sub> receptor isoform, PGF<sub>2α</sub> treatment led to retraction of filopodia, cell rounding, and a cobblestone appearance of the cells. This is demonstrated in Fig. 1. For these experiments, cells stably expressing the FP<sub>A</sub> receptor isoform (panels A and B), the FP<sub>B</sub> receptor isoform (panels C and D), or untransfected 293-EBNA cells (panels E and F) were plated at low density (~25,000 cells/well of a 12-well dish), grown for 2 to 3 days, and then unstimulated (panels A, C, and E) or stimulated with 1 μM PGF<sub>2α</sub> (panels B, D, and F) overnight at 37 °C. As shown in panels B and D, PGF<sub>2α</sub> treatment affected the morphology of the cells expressing the FP<sub>A</sub> and FP<sub>B</sub> receptor isoforms but had no effect on untransfected cells (panel F). In subsequent experiments, the PGF<sub>2α</sub>-induced changes in cell morphology were apparent within 15 min after exposure to 1 μM PGF<sub>2α</sub>, and were maximal within 2 h after treatment (data not shown).

To gain understanding into the mechanisms underlying the FP prostanoid receptor isoform-mediated changes in cell morphology, we examined whether known effectors of cellular activation were involved. The possibility of PKC involvement was suggested when it was observed that PMA also led to similar effects on the cells expressing the FPA receptor isoform (Fig. 1B, panel a). To determine whether both the PMA and PGF<sub>2α</sub>-induced changes in cell morphology were dependent upon activation of PKC we examined the ability of bisindolylmaleimide I (also known as GF109203X; Calbiochem) to inhibit the PGF<sub>2α</sub>- and PMA-induced changes in cell morphology. As demonstrated in Fig. 2A,
with cells expressing the FP\textsubscript{A} receptor isoform (panels a and b) or the FP\textsubscript{B} receptor isoform (panels c and d), treatment of the cells with 2.5 \(\mu\)M bisindolylmaleimide I alone had no effect on cell morphology (panels a and c). In addition, pretreating the cells for 15 min with 2.5 \(\mu\)M bisindolylmaleimide had no effect on the PGF\textsubscript{2\alpha}-induced changes in cell morphology in cells expressing either isoform (panels b and d). However, as shown in Fig. 2B, the PMA-induced changes in cell morphology could be prevented by pretreating the cells with bisindolylmaleimide I (panel b). In addition to the role for PKC, we also determined whether increases in intracellular calcium were involved in the PGF\textsubscript{2\alpha}-induced changes in cell morphology. However, doses of up to 2.5 \(\mu\)M BAPTA-AM, an intracellular calcium chelator, had no effect on the PGF\textsubscript{2\alpha}-induced changes in cell morphology (data not shown).

One of the intracellular signaling pathways that has been known to be involved in G-protein-coupled receptor-mediated changes in cell morphology is the activation of the small G-protein Rho (13, 14, 15). C3 exoenzyme, a bacterial toxin that ADP-ribosylates and thereby inactivates Rho, was used to test the potential involvement of Rho in the FP\textsubscript{A} and FP\textsubscript{B}-mediated changes in cell morphology. As shown in Fig. 3A for cells expressing the FP\textsubscript{A} isoform and in Fig. 3B for cells expressing the FP\textsubscript{B} isoform, cells were either unstimulated (panels a and b) or pretreated with 40 \(\mu\)g/ml C3 exoenzyme for 48 h (panels c and d). The cells were then either unstimulated (panels a and c) or stimulated with 100 nM PGF\textsubscript{2\alpha}, for 1 h (panels b and d). Pretreatment of the cells with C3 exoenzyme almost completely inhibited the PGF\textsubscript{2\alpha}-induced changes in cell morphology, suggesting the involvement of Rho in the FP\textsubscript{A} and FP\textsubscript{B} receptor-mediated changes in cell morphology.

In many systems, activation of Rho leads to the formation of actin stress fibers (16, 17). Since our data suggested that the FP\textsubscript{A} and FP\textsubscript{B}-mediated changes in cell morphology were Rho-dependent, we examined the possibility that activation of either isoform would also promote the formation of Rho-mediated actin stress fibers. As shown in Fig. 4A for cells expressing the FP\textsubscript{A} receptor isoform and in Fig. 4B for cells expressing the FP\textsubscript{B} receptor isoform, cells were either untreated (panels a and b) or pretreated with 40 \(\mu\)g/ml C3 exoenzyme for 48 h (panels c and d). The cells were then either unstimulated (panels a and c) or stimulated with 100 nM PGF\textsubscript{2\alpha}, for 1 h (panels b and d). Cells were then fixed, phalloidin-stained, and imaged as described under “Experimental Procedures.” As shown in panel b, PGF\textsubscript{2\alpha} treatment of cells stably expressing the FP\textsubscript{A} or FP\textsubscript{B} receptor isoforms led to the formation of actin stress fibers that were not present in untreated cells (panel a). Pretreatment of the cells with C3 exoenzyme abrogated the PGE\textsubscript{2\alpha}-induced formation of actin stress fibers (panel d), suggesting that Rho is essential for the FP receptor-mediated formation of actin stress fibers in these cells.

One of the pathways that has previously been shown to be activated by PGE\textsubscript{2\alpha} is the tyrosine phosphorylation of p125...
focal adhesion kinase (FAK) (5, 18). In several systems, the tyrosine phosphorylation of p125 FAK has been shown to be blocked by C3 exoenzyme, suggesting that it is downstream of Rho activation (19, 20). Thus, we wanted to determine if activation of FP receptors would lead C3 exoenzyme-sensitive phosphorylation of p125 FAK. As shown in Fig. 5, FPA-expressing cells were either untreated (panel A) or pretreated with 40 μg/ml C3 exoenzyme for 48 h (panel B). The cells were then either unstimulated (panels a and c) or stimulated for 1 h with 100 nM PGF2α (panels b and d). The cells were then imaged as described under “Experimental Procedures.” Images in A and panels a and b of B were obtained at a magnification of 66×, whereas those in panels c and d of B were obtained at 75×. The pictures are representative of at least three independent experiments.

FIG. 3. The effect of C3 exoenzyme on the PGF2α-induced changes in cell morphology in 293-EBNA cells that stably express the FPA or FPB prostanoid receptor isoforms. Cells transfected with the FPA (A) or FPB (B) were plated at a density of 25,000 cells/well into 12-well dishes and untreated (panels a and b) or pretreated for 48 h with 40 μg/ml C3 exoenzyme (panels c and d). The cells were then either unstimulated (panels a and c) or stimulated for 1 h with 100 nM PGF2α (panels b and d). The cells were then imaged as described under “Experimental Procedures.” Images in A and panels a and b of B were obtained at a magnification of 66×, whereas those in panels c and d of B were obtained at 75×. The pictures are representative of at least three independent experiments.

FIG. 4. Effect of C3 exoenzyme on PGF2α-induced formation of actin stress fibers in 293-EBNA cells stably expressing the FPA (A) and FPB (B) prostanoid receptor isoforms. Cells were plated at low density on glass coverslips in 6-well dishes for 1 day. The cells were either not treated (panels a and b) or were pretreated with 40 μg/ml C3 exoenzyme for 48 h (panels c and d). The cells were then either unstimulated (panels a and c) or stimulated for 1 h with 100 nM PGF2α (panels b and d). The cells were fixed and stained with phalloidin as described under “Experimental Procedures.” The images in A were obtained at a magnification of 330×; whereas those in B were obtained at 225×. These images are representative of three independent experiments.

focal adhesion kinase (FAK) (5, 18). In several systems, the tyrosine phosphorylation of p125 FAK has been shown to be blocked by C3 exoenzyme, suggesting that it is downstream of Rho activation (19, 20). Thus, we wanted to determine if activation of FP receptors would lead C3 exoenzyme-sensitive phosphorylation of p125 FAK. As shown in Fig. 5, FPA-expressing cells were either untreated (panel A) or pretreated with 40 μg/ml C3 exoenzyme for 48 h (panel B). The cells were then either unstimulated (panels a and c) or stimulated for 1 h with 100 nM PGF2α (panels b and d). The cells were then imaged as described under “Experimental Procedures.” Images in A and panels a and b of B were obtained at a magnification of 66×, whereas those in panels c and d of B were obtained at 75×. The pictures are representative of at least three independent experiments.

**FIG. 3.** The effect of C3 exoenzyme on the PGF2α-induced changes in cell morphology in 293-EBNA cells that stably express the FPA or FPB prostanoid receptor isoforms. Cells transfected with the FPA (A) or FPB (B) were plated at a density of 25,000 cells/well into 12-well dishes and untreated (panels a and b) or pretreated for 48 h with 40 μg/ml C3 exoenzyme (panels c and d). The cells were then either unstimulated (panels a and c) or stimulated for 1 h with 100 nM PGF2α (panels b and d). The cells were then imaged as described under “Experimental Procedures.” Images in A and panels a and b of B were obtained at a magnification of 66×, whereas those in panels c and d of B were obtained at 75×. The pictures are representative of at least three independent experiments.

**FIG. 4.** Effect of C3 exoenzyme on PGF2α-induced formation of actin stress fibers in 293-EBNA cells stably expressing the FPA (A) and FPB (B) prostanoid receptor isoforms. Cells were plated at low density on glass coverslips in 6-well dishes for 1 day. The cells were either not treated (panels a and b) or were pretreated with 40 μg/ml C3 exoenzyme for 48 h (panels c and d). The cells were then either unstimulated (panels a and c) or stimulated for 1 h with 100 nM PGF2α (panels b and d). The cells were fixed and stained with phalloidin as described under “Experimental Procedures.” The images in A were obtained at a magnification of 330×; whereas those in B were obtained at 225×. These images are representative of three independent experiments.

**DISCUSSION**

Here we have demonstrated that activation of the cloned ovine FPA and FPB prostanoid receptor isoforms stably expressed in 293-EBNA cells leads to Rho-dependent changes in cell morphology, formation of actin stress fibers, and tyrosine phosphorylation of p125 FAK. Although the FP receptor is known to be coupled to stimulation of intracellular calcium release and protein kinase C activation via the G-protein Gq, the Rho-dependent changes in cell morphology appear to be independent of either of these two effectors. This suggests that both the FPA and FPB prostanoid receptor isoforms can couple
FP Prostanoid Receptors Activate Rho

FIG. 5. The effect of C3 exoenzyme on the PGF\textsubscript{2α}-stimulated tyrosine phosphorylation of p125 FAK in 293-EBNA cells stably expressing the FP\textsubscript{A} prostanoid receptor isoform. 293-EBNA cells that stably express the FP\textsubscript{A} receptor isoform were serum-starved for 48 h in the absence (panel A) or presence (panel B) of 40 μg/ml C3 exoenzyme. The cells were then stimulated for the indicated times with 1 μM PGF\textsubscript{2α}. The cells were lysed and immunoprecipitated with an anti-FAK-specific antibody (C-20, Santa Cruz Biochemicals), and Western blotting was performed as indicated under “Experimental Procedures” using an antiphosphotyrosine-specific antibody (PY-20, Transduction labs). To ensure equal loading, the Western blots were stripped and re-probed using the anti-FAK-specific antibody. This experiment is representative of one of three independent experiments.

There are several examples of other G-protein-coupled receptors that are able to couple to a classical G-protein-coupled pathway (G\textsubscript{q} or G\textsubscript{i}) and to a separate Rho-dependent pathway. For instance, the thrombin receptor, like the FP\textsubscript{A} prostanoid receptor isoform, is a G\textsubscript{q}-coupled receptor that stimulates changes in cell morphology that are independent of PKC activation and intracellular calcium release and are pertussis toxin-insensitive (14). Like the FP\textsubscript{A} receptor isoform, the changes in cell morphology induced by thrombin are sensitive to C3 exoenzyme, demonstrating that the changes in cell morphology are Rho-dependent (14).

Among the prostanoid receptors, activation of the G\textsubscript{i}-coupled EP\textsubscript{3} prostanoid receptor expressed in PC-12 cells led to the Rho-dependent retraction of nerve growth factor-factor-stimulated neurite outgrowth (13). Like the FP\textsubscript{A} receptor and thrombin-induced changes in cell morphology, the EP\textsubscript{3}-mediated changes were insensitive to PKC inhibitors and intracellular calcium chelators but were sensitive to C3 exoenzyme (13). Interestingly, differences have been found between two EP\textsubscript{3} receptor isoforms in their ability to activate Rho (21). Like the FP\textsubscript{A} receptor isoforms, the EP\textsubscript{3} receptor isoforms differ only in their carboxyl termini. It was recently reported that in Madin-Darby canine kidney cells expression of the EP\textsubscript{3}-isoform led to the constitutive formation of actin stress fibers, whereas in cells expressing the EP\textsubscript{3} isoform, stress fibers were formed in an agonist-dependent manner (21). The data with the EP\textsubscript{3} receptor isoforms suggest that there are regions in the carboxy termini that may be important in modulating the interaction with the effectors involved in stress fiber formation. With the FP receptor isoforms, however, it does not appear that the longer FP\textsubscript{A} carboxyl terminus has a role in mediating the effects of PGF\textsubscript{2α} on shape change, stress fiber formation, and p125 FAK phosphorylation. Most likely, the regions of the FP\textsubscript{A} receptor isoform that are not present in the FP\textsubscript{B} regulate receptor-effector interactions in other ways, such as regulating desensitization.

Although we have demonstrated that Rho is involved in the FP\textsubscript{A} and FP\textsubscript{B} receptor-mediated changes in cell morphology, the pathway leading from the receptor to Rho remains unknown. However, one potential pathway involves the heterotrimeric G-proteins G\textsubscript{12} and G\textsubscript{13}. Although the immediate effectors of these two G-proteins are not well characterized, previous studies have demonstrated that direct injection of constitutively active forms of either of these G-proteins into Swiss 3T3 cells induced the formation of actin stress fibers that was blocked by the injection of C3 exoenzyme (22). G\textsubscript{12} has also been implicated in the Rho-dependent formation of actin stress fibers induced by lysophosphatidic acid in Swiss 3T3 cells (23). Besides the formation of actin stress fibers, G\textsubscript{12} and G\textsubscript{13} have also been implicated in the Rho-mediated tyrosine phosphorylation of p125 FAK (20). Specifically, transient overexpression of constitutively active mutants of either G\textsubscript{12} or G\textsubscript{13} led to tyrosine phosphorylation of p125 FAK, which was blocked by co-transfection with a plasmid encoding C3 exoenzyme (20). Since the present findings indicate that the FP receptor isoforms couple to the Rho-dependent formation of actin stress fibers, the heterotrimeric G-proteins G\textsubscript{12} and G\textsubscript{13} are likely effectors.

Here we have demonstrated that both the FP\textsubscript{A} and FP\textsubscript{B} prostanoid receptor isoforms are coupled to the activation of Rho. In addition to demonstrating for the first time that the FP receptor isoforms both couple to Rho-dependent pathways, these data suggest that other regions of the receptor besides the carboxyl termini are responsible for the interaction with the Rho-dependent effectors. The identification of Rho as an effector of the FP receptor isoforms will give clues to some of the physiological effects of PGF\textsubscript{2α}, such as the PGF\textsubscript{2α}-induced hypertyrose of rat-isolated cardiomycocytes (5) and the PGF\textsubscript{2α}-induced in vivo cardiac growth (3).

Acknowledgments—We thank Dr. Richard Vaillancourt (University of Arizona) for helpful discussions and assistance, Dr. Alan Hall (University College, London) for the plasmid encoding C3 exoenzyme, and Angela Guevara for technical assistance with the C3 exoenzyme purification.

REFERENCES
1. McCracken J. A., Glew, M. E., and Scaramuzzi, R. J. (1970) J. Clin. Endocrino-

Metab. 30, 544–546
2. Camras, C. B., Schmer, R. A., Marsk. A., Lastgarten J. S., Serle, J. B.,
Sternschacht, J., Bito, L. Z., and Podos, S. M. (1990) Arch. Ophthalmol.
110, 1735–1738
3. Lai, J., Jin, H., Yang, B., Winer, J., Li, W., Yen, R., King, K. L., Zeigler, F., Ko,
A., Cheng, J., Bunting, S., and Paeni N. F. (1996) An. J. Physiol.
271, H2197–H2208
4. Kumanpili, P., Lawson, J. A., Rokach, J. A., Meinkeith, J. L., and FitzGerald,
G. A. (1998) J. Biol. Chem. 273, 22442–22452
5. Adams, J. W., Sah, V. P., Henderson, S. A., and Brown, J. H. (1998) Circ. Res.
73, 167–176
6. Adams, J. W., Migita, D. S., Yu, M. K., Young, R., Helleckson, M. S., Castro-
Vargas, P. E., Domingo, J. D., Lee, P. H., Bui J. S., and Henderson, S. A.
(1996) J. Biol. Chem. 271, 1179–1186
7. Abramovitz, M., Boie, Y., Nguyen, T., Rushmore, T. H., Bayne, M. A., Metters,
K. M., Slpetz, D. M., and Grynszczak, R. (1994) J. Biol. Chem. 269,
2632–2836
8. Sugimoto, Y., Hasumoto, K., Namba, T., Irie, A., Katsuyama, M., Negishi, M.,
Kakizuka, A., Nariyama, S., and Ichikawa, A. (1994) J. Biol. Chem.
269, 1356–1360
9. Sakamoto, K., Ezashi, T., Miwa, K., Okuda-Ashitaka, E., Houtami, T.,
Sugimoto, T., Ito, H., and Hayashi, O. (1994) J. Biol. Chem. 269, 3881–3886
10. Graves, P. E., Pierce, K. L., Bailey, T. J., Rueda, B. R., Gil, D. W., Woodward,
D. F., Yool, A. J., Hoyer, P. B., and Regan, J. W. (1995) Endocrinology
136, 3430–3436
11. Pierce, K. L., Bailey, T. J., Hoyer, P. B., Gil, D. W., Woodward, D. F.,
Yool, A. J., Hoyer, P. B., and Regan, J. W. (1997) J. Biol. Chem.
272, 883–887
12. Dillon S. T., and Feig L. A. (1995) Methods Enzymol. 256, 174–184
13. Katoh, H., Negishi, M., and Ichikawa, A. (1996) J. Biol. Chem. 271,
29780–29784
14. Majumdar, M., Seasholtz, T. M., Goldstein, D., de Lanorelle, P., and Brown, J. H. (1998) J. Biol. Chem. 273, 10099–10106
15. Lee, M.-J., Van Brocklyn, J. R., Thangada, S., Liu, C. H., Hand, A. R., Menzellev, R., Spiegel, S., and Hla, T. (1998) Science 279, 1552–1555
16. Hall, A. (1998) Science 279, 509–514
17. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
18. Watanabe, T., Nakao, A., Emerling, D., Hashimoto, Y., Tsukamoto, K., Horie, Y., Kinoshita, M., and Kurokawa, K. (1994) J. Biol. Chem. 269, 17619–17625
19. Béting, S., Daviaud, D., Pagès, C., Bonnard, E., Valet, P., Lafontan, M., and Saulnier-Blache, J. S. (1998) J. Biol. Chem. 273, 15804–15810
20. Needham, L. K., and Rozengurt, E. (1998) J. Biol. Chem. 273, 14626–14632
21. Hasegawa, H., Negishi, M., Katoh, H., and Ichikawa, A. (1997) Biochem. Biophys. Res. Comm 234, 631–636
22. Buhl, A. M., Johnson, N. L., Dhanasekaran N., and Johnson, G. L. (1995) J. Biol. Chem. 270, 24631–24634
23. Gohla, A., Harhammer, R., and Schultz, G. (1998) J. Biol. Chem. 273, 4653–4659