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IDENTIFICATION OF A SINGLE (FP) RECEPTOR ASSOCIATED WITH PROSTANOID-INDUCED Ca^{2+} SIGNALS IN SWISS 3T3 CELLS

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Abstract—Thus far, the prostanoid FP-receptor has been characterized only on the basis of agonist studies. It is currently classified as a receptor having particular sensitivity to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) but with the ability to recognize prostaglandins D_2 and E_2 (PGD_2 and PGE_2). We have re-examined this concept by studying second messenger Ca^{2+} signals to $PGF_{2\alpha}$, PGD_2 and PGE_2, and performing radioligand binding studies in Swiss 3T3 cells. The same rank order of potency was obtained for both the Ca^{2+} transient signal and competition for $PGF_{2\alpha}$ binding sites. The potency rank order, $PGF_{2\alpha} > PGD_2 > PGE_2$, was identical to that obtained from functional studies in isolated tissues, such as the cat iris. Additional support for the concept that $PGF_{2\alpha}$, PGD_2, and PGE_2 interact with a single receptor to elicit a Ca^{2+} signal was provided by successive addition studies. Thus, cells pretreated with a supramaximal concentration of $PGF_{2\alpha}$ exhibited little or no response to subsequent administration of PGD_2 or PGE_2. Likewise, cells pretreated with a large concentration of PGD_2 or PGE_2 exhibited minimal responsiveness to successive addition of the corresponding alternative prostaglandins. Pretreatment with a maximally effective concentration of $PGF_{2\alpha}$, PGD_2, or PGE_2 rendered the cells refractory to the FP-receptor selective agonist fluprostenol, which further supports the hypothesis that Ca^{2+} transient signals in response to prostanoids in Swiss 3T3 cells are mediated by the FP-receptor. The Ca^{2+} transient responses to $PGF_{2\alpha}$, PGD_2, and PGE_2 also exhibited a similar modest reduction when extracellular Ca^{2+} was removed. Finally, the DP-receptor antagonist BW A868C did not block the Ca^{2+} transient response to PGD_2, indicating an absence of DP-receptor involvement. Moreover, Ca^{2+} responses to the thromboxane A_2 mimetic U-46619 were unaffected by the TP-antagonist BM 13505, which indicates no involvement of the TP-receptor. These results support the contention that the FP-receptor has particular sensitivity to $PGF_{2\alpha}$ but will also recognize PGD_2 and PGE_2.

Key words: prostaglandins; receptors; fibroblasts; calcium; binding; Fura-2

The contention that distinct receptor subtypes exist for prostanoids has received increased acceptance in recent years, and the working classification for prostanoid receptors [1, 2] is now widely employed. Different receptors for prostaglandins were originally proposed according to the rank order of potency of natural prostanoids in certain tissues [1–3] and the selective activity of synthetic prostanoids [1, 2, 4]. The existence of certain prostanoid receptors has been supported by studies with selective antagonists. Thus, antagonists for the DP-receptor [5], the EP_1-receptor and the TP-receptor [1, 6, 7] have been instrumental in defining these receptor subtypes. Moreover, cDNA for the TP-receptor [8] and the EP_3-receptor [9] have been obtained recently. In contrast, the definition of other prostanoid receptors such, as FP-, and IP-receptors, remains limited to functional activity in response to agonists.

The ability of $PGF_{2\alpha}$§ and its synthetic analogs to lower intraocular pressure in a variety of mammalian species, including humans [10, 11], indicates the therapeutic importance of the FP-receptor as a target for anti-glaucoma prostanoids. $PGF_{2\alpha}$ also potently stimulates DNA synthesis and cell proliferation in fibroblasts [12, 13]. Despite its potential therapeutic importance, definition of the prostanoid FP-receptor to date is confined to the apparent selectivity of a few agonists in isolated tissue preparations and the functional rank order of potency of natural prostaglandins. The purpose of these studies was to determine if PGD_2 and PGE_2 exert their effects in biological systems with particular sensitivity to $PGF_{2\alpha}$ by stimulating a single receptor subtype, as proposed by the current classification [1, 2]. This was achieved by comparing the second messenger Ca^{2+} signal and radioligand binding in a single cell system. This enabled characterization of the FP-receptor to be extended within the limits imposed by the currently available pharmacological agents. Mouse Swiss 3T3 fibroblasts were employed for the present studies since, unlike isolated tissue preparations, the biochemical events underlying functional responses to $PGF_{2\alpha}$ are well characterized in murine fibroblasts. Thus, $PGF_{2\alpha}$ stimulates PI

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§ Abbreviations: $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGD_2 prostaglandin D_2; PGE_2, prostaglandin E_2; PI, phosphoinositide; DAG, diacylgycerol; Ins 1,4,5 P_3, inositol 1,4,5-trisphosphate; PDGF, platelet-derived growth factor; DMEM, Dulbecco's modified Eagle's medium; Fura-2AM, fura-2 acetoxymethyl ester; and $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

hydrolysis with the resultant generation of DAG and Ins 1,4,5 P_3 [14]. The liberation of Ca^{2+} from intracellular stores induced by Ins 1,4,5 P_3 [15] was of particular value for analyzing functional responses to prostanoids since Ca^{2+} signalling can be monitored continuously. Continuous monitoring of intracellular Ca^{2+} uniquely allows receptor occupation to be related to functional responsiveness by successive addition studies.

MATERIALS AND METHODS

Materials. PGD_2, PGE_2, $PGF_{2\alpha}$, and 9,11-dideoxy-9_α,11_α-methanoepoxy-$PGF_{2\alpha}$ (U-46619) were purchased from Cayman Chemical, Ann Arbor, MI. BW A868C and BW 245C (Burroughs Wellcome, Beckenham, U.K.) were gifts; fluprostenol was supplied by Cooper (Berkhamsted, England). Prostaglandin solutions were prepared by adding 2% Na_2CO_3 with subsequent neutralization by 0.1 N HCl. BW A868C was prepared as a stock solution in ethanol. BM 13505 (Daltroban), a gift from Boehringer-Mannheim (Mannheim, Germany), was also dissolved in Na_2CO_3, and the pH was adjusted to 8.5. PDGF was purchased from the Sigma Chemical Co. (St. Louis, MO), and stock solutions were prepared by adding 1 N acetic acid. Ionomycin was also purchased from Sigma. Radiolabeled $PGF_{2\alpha}$ [5,6,8,9,11,12,14,15-^{3}H(N)] was purchased from New England Nuclear (Wilmington, DE).

Cell culture. Mouse Swiss 3T3 fibroblasts were plated in culture flasks and were fed DMEM containing 10% fetal bovine serum, 2 mM L-glutamine, and 0.05 mg/mL gentacin (all were purchased from GIBCO, Grand Island, NY). Cell cultures were maintained in a humidified atmosphere of 95% air, 5% CO_2 and grown to confluency.

Determination of $[Ca^{2+}]$. Measurement of $[Ca^{2+}]$ was achieved by incorporating the Ca^{2+}-sensitive fluorescent probe Fura-2AM into cells in suspension. Cells were removed from the culture flask by approximately 1-min treatment with trypsin 0.05%/0.53 mM EDTA (GIBCO) at 37°. Proteolytic activity was arrested by adding 5 mL of 10% fetal bovine serum in DMEM. The cells were consecutively washed in Hanks' balanced salt solution; centrifugation for the washes was performed for 15 min at 200 *g* at room temperature. Cells were counted, resuspended in buffer containing 140 mM NaCl, 50 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM HEPES: Tris 7.4, 5 mM glucose, 5 mM sodium pyruvate, and bovine serum albumin (0.1%), and incubated with 2 μM Fura 2AM in a shaking water bath for 30 min at 37°. The cells were subsequently washed in buffer containing 120 mM NaCl, 6 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgSO_4$, 20 mM HEPES, 1 mg/mL glucose, 1 mg/mL sodium pyruvate adjusted to pH 7.4 with NaOH and resuspended at a concentration of 2×10^6 cells/mL. Aliquots of 0.5 mL cell suspension were then added to autocap microtubes to provide 10^6 cells per experimental determination of $[Ca^{2+}]_i$.

Fluorescence was measured in a Perkin–Elmer LS-5 fluorescence spectrophotometer at excitation and emission wavelengths of 340 and 492 nm, respectively, with both slits at 10 nm. For each experimental determination, 10^6 cells were washed in the above buffer, centrifuged at 200 *g* for 5 min and resuspended in a cuvette. For studies in Ca^{2+}-free buffer, each cuvette also contained 0.4 mM EGTA. Stirring was achieved by an overhead-mounted, paddle stirrer with the temperature maintained at 37°. Calibration of the Fura 2 signal was as described previously for Quin 2 and Fura 2 [16, 17]. The cells were lysed with digitonin (10 μL × 100 mg/mL: in DMSO) to obtain maximum fluorescence. EGTA (100 mM) and sufficient 10 N NaOH to adjust the pH to 8.5 were then added successively to obtain minimum fluorescence.

Preparation of membranes. Cells were removed from culture flasks by scraping, were suspended in 50 mL Hanks' medium, and were centrifuged at 6500 *g* for 10 min. The pellet was suspended in 50 mL of ice-cold 0.32 M sucrose–50 mM Tris buffer at pH 7.4 and centrifuged as above. This pellet was then resuspended in 40 mL of sucrose–Tris buffer and homogenized with a Polytron homogenizer for 1 sec at setting 8. The homogenate was centrifuged at 400 *g* for 5 min, and the supernatant was removed with a Pasteur pipette and saved. The residue was resuspended in 25 mL of buffer, homogenized and centrifuged as before. The resulting pooled supernatant was then centrifuged at 177,000 *g* for 40 min. This crude membrane pellet was suspended over a sucrose cushion, 1.06 M providing optimal specific binding. Four milliliters of homogenate was layered on top of 4 mL of 1.06 M sucrose contained in six (10 mL) Beckman polycarbonate centrifuge tubes, and centrifuged at 112,000 *g* for 1.5 hr. The top band separating the interface of the 1.06 M sucrose cushion and the subcellular suspension was enriched with plasma membranes (PM). The PM bands from each of the tubes were aspirated with a Pasteur pipette, combined and diluted 1:2 with 50 mM Tris buffer. They were then centrifuged at 304,000 *g* for 40 min. The final pellet was washed once with Tris–HCl buffer and stored at −70°.

Radioligand binding studies. For the radioligand competition studies, each tube contained 3 mM $CaCl_2$, 5 nM [^{3}H]$PGF_{2\alpha}$ 50–100 μg protein and graded concentrations of unlabeled $PGF_{2\alpha}$, PGD_2, PGE_2 or BW 245C in 50 mM Tris–HCl buffer at pH 7.4 to provide a final volume of 200 μL. The binding reaction was started by adding resuspended plasma membrane. Non-specific binding was determined using 10 μM unlabeled $PGF_{2\alpha}$. Incubation was terminated after 30 min by the addition of 4 mL of ice-cold Tris–HCl buffer and vacuum filtration through Whatman GF/B glass fiber filters using a Brandel cell harvester. Filters were rinsed three times with ice-cold Tris–HCl buffer and were oven dried for 1–2 hr. Dry filters were placed in 5 mL of Packard Emulsifier-safe scintillation fluid and counted on a Packard 1900 CA counter. The descriptions for receptor-enriched plasma membrane fractions and the radioligand binding assay describe the optimal procedures that were attained after a series of exploratory experiments. The results of these preliminary experiments are reported briefly in the relevant section.

RESULTS

The effects of the natural prostanoids PGD_2,

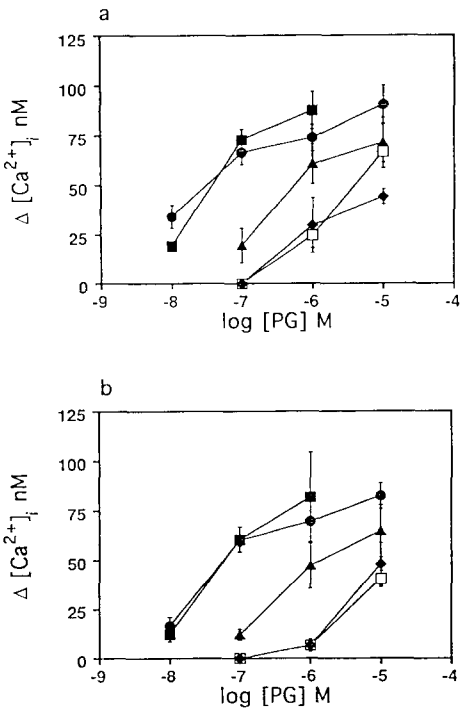

Fig. 1. Comparison of the effects of fluprostenol (■), $PGF_{2\alpha}$ (●), PGD_2 (▲), PGE_2 (◆) and U-46619 (□) in the presence (a) and absence (b), of 1.4 mM $CaCl_2$ on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Swiss 3T3 cells. Points represent mean values ± SEM, N = 3.

PGE_2, and $PGF_{2\alpha}$, the FP-agonist fluprostenol and the TP-agonist U-46619 on $[Ca^{2+}]_i$ of Swiss 3T3 cells suspended in either medium containing 1.4 mM $CaCl_2$ or Ca^{2+}-free buffer containing 0.4 mM EGTA are depicted in Fig. 1. A concentration-dependent Ca^{2+} transient with the following rank order of potency: fluprostenol $\geq PGF_{2\alpha} > PGD_2 > PGE_2 \geq$ U-46619 was obtained (Fig.1). Removal of Ca^{2+} from the medium reduced, but did not abolish, Ca^{2+} transients elicited by each prostanoid, and this was observed as a small depression of the Ca^{2+} transient response for each concentration examined (Fig. 1b). The Ca^{2+} transient concentration–response curves to all prostanoids appeared to be similarly attenuated by removal of extracellular Ca^{2+} with the possible exception that the PGE_2 response was somewhat more affected. All prostanoids caused a rapid, initial increase in $[Ca^{2+}]_i$ which, in Ca^{2+}-containing medium, declined within 1–2 min to equilibrate at a new, slightly elevated basal level. Since there was an apparent gradual decline in baseline $[Ca^{2+}]_i$ in cells suspended in Ca^{2+}-free medium, it is difficult to draw reliable inferences regarding the post-transient basal $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. The effect of PDGF (10 ng/mL) in the presence and absence of extracellular Ca^{2+} was also examined, and the values were 65.4 ± 3.4 and 63.6 ± 2.9 mM, respectively. Ionomycin produced a response of 126 ± 6 nM in Ca^{2+}-free buffer, indicating that prostanoids and PDGF only partially liberate intracellular Ca^{2+} pools.

Sequential addition studies were performed to provide indirect evidence pertaining to the number of possible receptor subtypes involved in prostaglandin-induced Ca^{2+} transients. In these studies, cells were pretreated with a concentration of each prostaglandin selected to cause a maximal response, and the $[Ca^{2+}]_i$ was allowed to re-equilibrate to a new basal level. Subsequently, a cumulative concentration–response experiment was performed in the presence of the prostanoid originally added. In cells pre-stimulated with $PGF_{2\alpha}$ (10^{-6} M), successive addition of graded concentrations of PGD_2 (10^{-7}–10^{-5} M) or PGE_2 (10^{-6}–10^{-4} M) failed to produce a Ca^{2+} transient (Fig. 2).

Cells pre-stimulated with a maximally effective concentration of PGD_2 (10^{-5} M) or PGE_2 (10^{-4} M) were refractory to subsequent addition of $PGF_{2\alpha}$ (10^{-8}–10^{-6} M), and cells pretreated with PGD_2 (10^{-5} M) did not respond to subsequent additions of PGE_2, and vice versa. Representative examples from these studies are depicted in Fig. 2. Cells pretreated with a maximally effective concentration (10^{-6} M) of the selective FP-receptor agonist fluprostenol were rendered unresponsive to subsequent additions of $PGF_{2\alpha}$, PGD_2, or PGE_2 (Fig. 3). Conversely, fluprostenol was ineffective following pretreatment with $PGF_{2\alpha}$, PGD_2 or PGE_2 (representative traces, Fig. 3). Although cells did not respond to prostanoids after pre-stimulation with a maximally effective concentration of $PGF_{2\alpha}$, PGD_2, or PGE_2, the response to PDGF was affected minimally by prostanoid pretreatment. Thus, the magnitude of the Ca^{2+} transient evoked by PDGF was virtually identical in the presence or absence of a maximally effective concentration of $PGF_{2\alpha}$, PGD_2, or PGE_2 (representative traces, Fig. 4). The Ca^{2+} response to PGD_3 was unaffected by pretreatment with the DP receptor antagonist BW A868C (Fig. 5) and the response to the thromboxane A_2 mimetic U-46619 was not inhibited by pretreatment with a TP-antagonist BM 13505 (Fig. 6). Antagonists were given as a 5 min pretreatment.

The radioligand binding experiments required a series of preliminary studies to optimize the conditions, the results of which are briefly described as follows. The use of a sucrose cushion was necessary as experiments on the 177,000 *g* pellet yielded specific binding that did not exceed 15%. When the 177,000 *g* pellet was resuspended in homogenization buffer and layered over 0.84 M or 1.06 M sucrose cushions, specific binding values of 24 and 35%, respectively, were obtained using 6.1 nM [^{3}H] $PGF_{2\alpha}$ and 10 μM unlabeled $PGF_{2\alpha}$ to determine non-specific binding. The time course for $PGF_{2\alpha}$ binding was then studied over 2 hr at three different temperatures. Minimal binding was obtained at 4°. At 37°, the level of specific binding reached 23% by 15 min but declined thereafter to only 10%. A similar phenomenon was reported previously in corporal luteal $PGF_{2\alpha}$ radioligand binding studies at 37° [18]. $PGF_{2\alpha}$ specific binding at 23° reached a plateau at 30 min and remained at 40 ± 2%. A third experiment revealed that 3 mM Ca^{2+} improved the degree of specific binding from 38 to 58%; 51% specific binding was

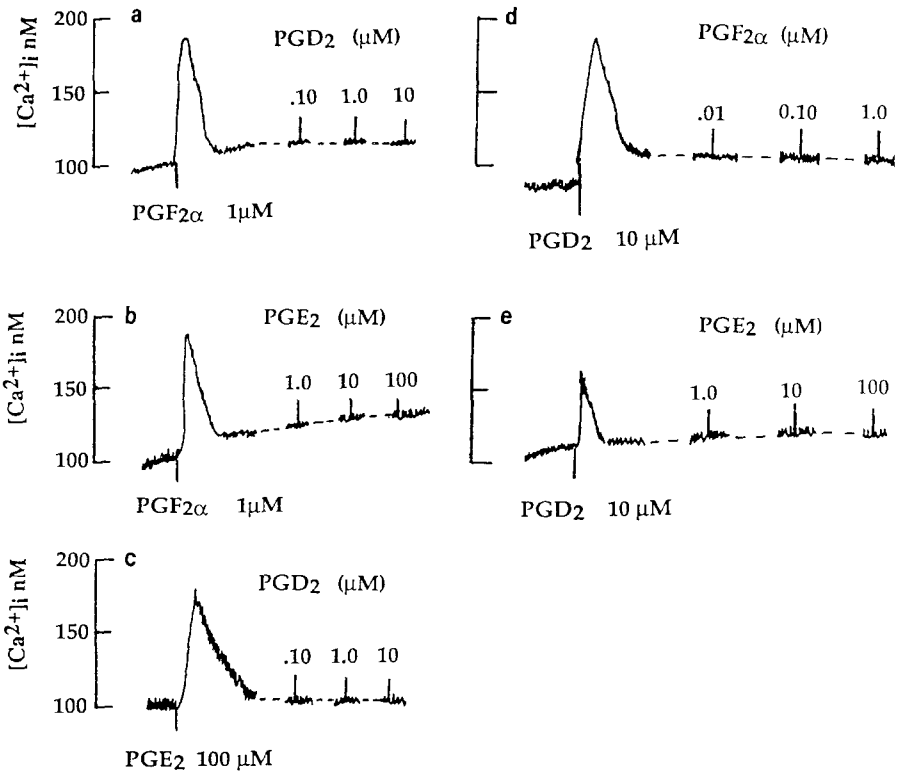

Fig. 2. Representative examples of successive addition studies with graded concentrations of (a) PGD_2 or (b) PGE_2 after pretreatment with 10^{-6} M $PGF_{2\alpha}$, (c) PGD_2 after 10^{-4} M PGE_2 pretreatment, and (d) $PGF_{2\alpha}$, or (e) PGE_2 after 10^{-5} M PGD_2 pretreatment. Prostaglandins were administered at 5 min intervals.

obtained with 100 mM NaCl. Thus, suitable conditions for binding studies were obtained.

The ability of graded concentrations of unlabeled $PGF_{2\alpha}$, PGD_2, and PGE_2 to compete with 5 nM [^{3}H] $PGF_{2\alpha}$ for its binding site under optimized experimental conditions is depicted in Fig. 7. The results of these competition studies revealed a rank order of inhibitory activity similar to the rank order of potency for eliciting a Ca^{2+} signal. Analysis of the competition curve for $PGF_{2\alpha}$ resulted in a Hill plot with a linear slope of 0.969. The IC_{50} (nM) values were as follows: $PGF_{2\alpha} = 24.8 \pm 3$; $PGD_2 = 581 \pm 85$; $PGE_2 = 3900 \pm 900$; and BW 245C = inactive.

DISCUSSION

Characterization of the prostanoid FP-receptor is currently based on the selectivity of synthetic analogues such as fluprostenol and a rank order of potency $PGF_{2\alpha} > PGD_2 > PGE_2 > PGI_2$ in isolated tissue preparations that are sensitive to $PGF_{2\alpha}$. Thus, in the cat and dog iris, particular sensitivity to fluprostenol and 17-phenyl $PGF_{2\alpha}$ is apparent and an ordinally descending level of potency is obtained with PGD_2 and PGE_2 [2, 19]. It has been suggested that PGD_2 and PGE_2 exert their effects by interacting with a single $PGF_{2\alpha}$-sensitive (FP-) receptor in $PGF_{2\alpha}$-sensitive tissues such as the cat and dog iris [2]. Radioligand binding studies in cell membrane preparations from the corpus luteum of farm animal species also indicate that natural prostaglandins compete with radiolabeled $PGF_{2\alpha}$ with the following rank order of potency: $PGF_{2\alpha} > PGD_2 > PGE_2$ [18, 20–23]. However, there is an absence of studies where the rank order of potency for functional responses or second messenger stimulation is directly compared with receptor binding studies in a single preparation. This implies that the alternative possibility of distinct receptor subtypes for PGD_2 and PGE_2 in $PGF_{2\alpha}$-sensitive preparations has not been adequately addressed.

Although interaction at a single $PGF_{2\alpha}$-sensitive receptor has been proposed to explain the activities of PGD_2 and PGE_2 in some preparations [2], alternative concepts have emerged. Thus, in osteoblast-like cells, independent receptors for each natural prostaglandin have been proposed in MC 3T3-E1 cells, and it was suggested that PGD_2 stimulates PI hydrolysis by a mechanism independent of $PGF_{2\alpha}$ and PGE_2-induced responses [24, 25]. In UMR-106 cells, PI hydrolysis and release of intracellular Ca^{2+} in response to $PGF_{2\alpha}$ and PGE_2 also appeared to involve different receptor subtypes according to successive addition studies [17]. Studies in mouse fibroblast cell lines involving similar

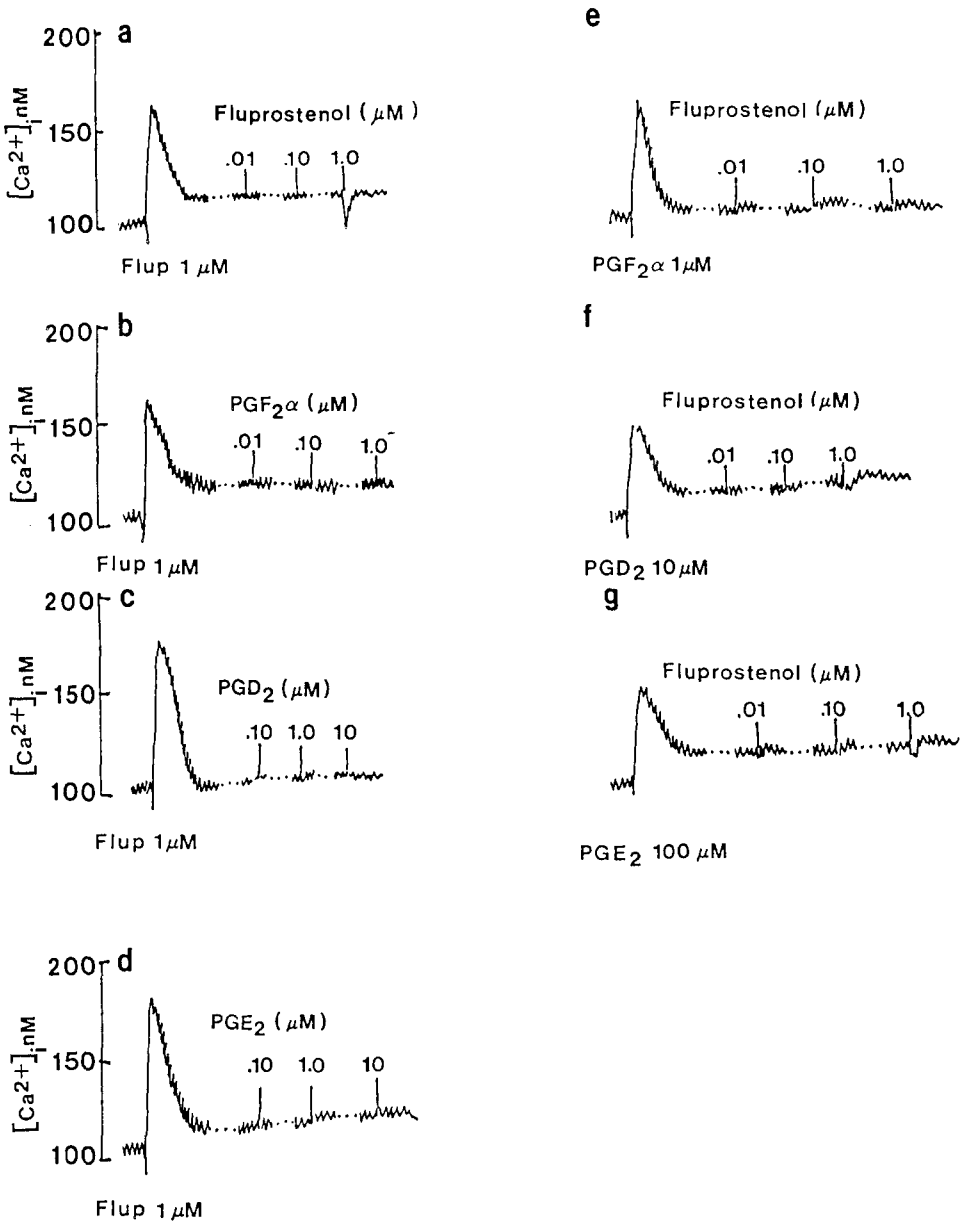

Fig. 3. Representative examples of successive addition studies with graded concentrations of (a) fluprostenol, (b) $PGF_{2\alpha}$, (c) PGD_2, and (d) PGE_2 on $[Ca^{2+}]_i$ after pretreatment with 10^{-6} M fluprostenol and successive addition of graded concentrations of fluprostenol after pretreatment with (e) 10^{-6} M $PGF_{2\alpha}$, (f) 10^{-5} M PGD_2, or (g) 10^{-4} M PGE_2. Administration of prostaglandins was at 5 min intervals.

manipulations of second messenger responses have, in contrast, indicated that PGD_2 and PGE_2 may be capable of interacting with a receptor sensitive to $PGF_{2\alpha}$ and the selective agonist fluprostenol [26, 27]. Studies that have attempted to link functional responses to natural prostaglandins with radioligand binding data appear to be restricted to the corpus luteum. When a comparison of the direct interaction of prostaglandins at the $PGF_{2\alpha}$ receptor in the bovine corpus luteum was made with luteolytic potency in heifers, only a limited association was reported [21]. Considering the fragmented evidence that has accumulated to date, the concept that the FP-receptor exists as a $PGF_{2\alpha}$-sensitive receptor that will also accept PGD_2 and PGE_2 as ligands and elicit a resultant biological response is not compelling.

In the present studies, we provide direct and indirect evidence that PGD_2 and PGE_2 can interact with and stimulate $PGF_{2\alpha}$-sensitive (FP) receptors. Both PGD_2 and PGE_2 were able to compete with $PGF_{2\alpha}$ for its receptor, and the rank order of potency for inhibition of $PGF_{2\alpha}$ binding was similar to that obtained for elicitation of a transient Ca^{2+} signal. Additional supportive evidence for single receptor

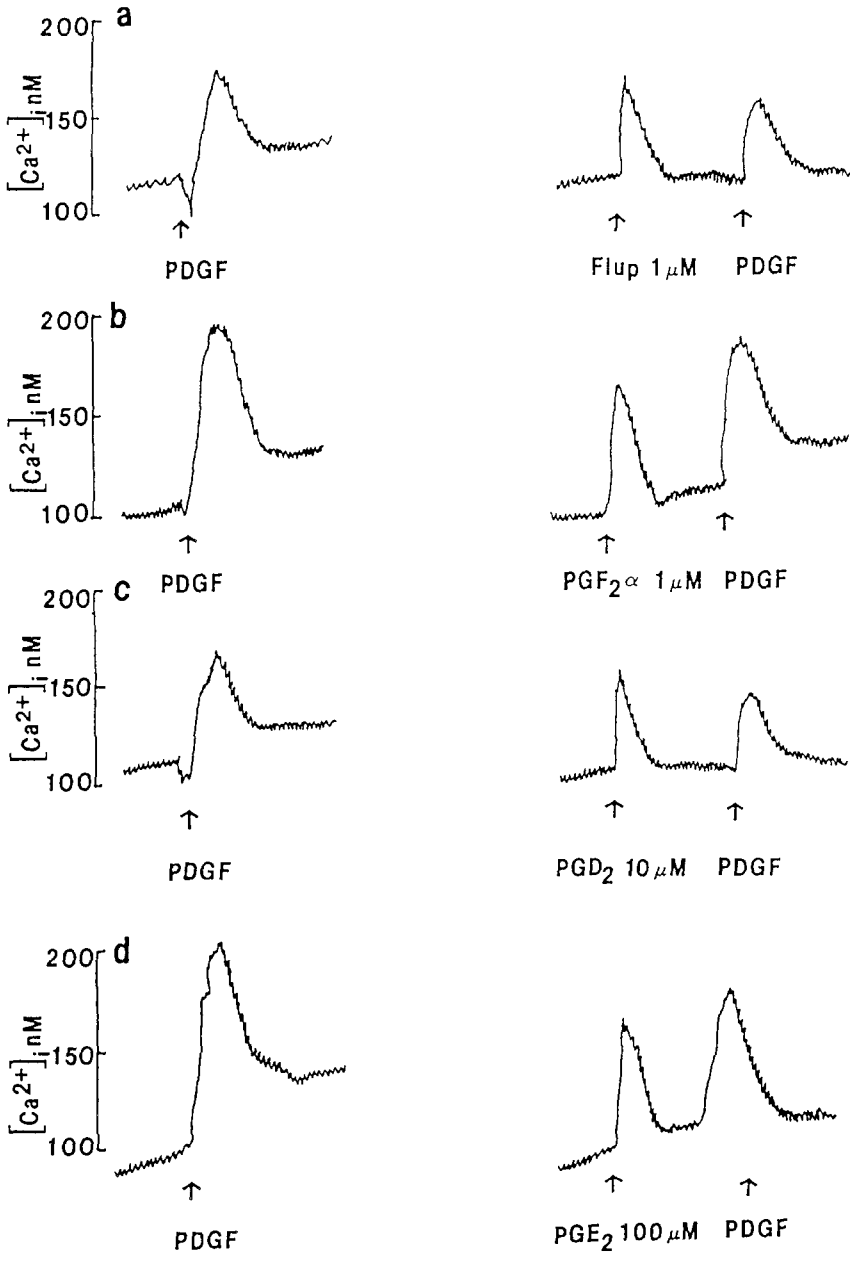

Fig. 4. Representative examples depicting the effect of PDGF (~10 ng/mL) on the $[Ca^{2+}]_i$ response in cells before and after pretreatment with (a) 10^{-6} M fluprostenol, (b) 10^{-6} M $PGF_{2\alpha}$, (c) 10^{-5} M PGD_2, or (d) 10^{-4} M PGE_2. PDGF was administered 5 min after each prostanoid.

involvement in the Ca^{2+} transient response was also obtained. Removal of extracellular Ca^{2+} affected the response to $PGF_{2\alpha}$, PGD_2, and PGE_2 in a similar manner, the largest component of the transient Ca^{2+} signal being released from an intracellular location. Pretreatment with a concentration of each prostaglandin sufficient to occupy most of the receptor sites rendered the cells refractory to stimulation by subsequent addition of PGD_2, PGE_2, or $PGF_{2\alpha}$. Thus, cells pretreated with a maximally effective concentration of $PGF_{2\alpha}$ did not respond to successive concentrations of PGD_2 or PGE_2. Likewise, cells pretreated with a large concentration of PGD_2 or PGE_2 exhibited little response to subsequent addition of the corresponding alternative prostaglandins. These results suggest that PGD_2, PGE_2, and $PGF_{2\alpha}$ all stimulate a single population of receptors. The contention that pretreatment with a large concentration of each prostaglandin occupies the majority of receptors, leaving insufficient binding sites available for successive prostaglandin additions, is consistent with the binding data. The single

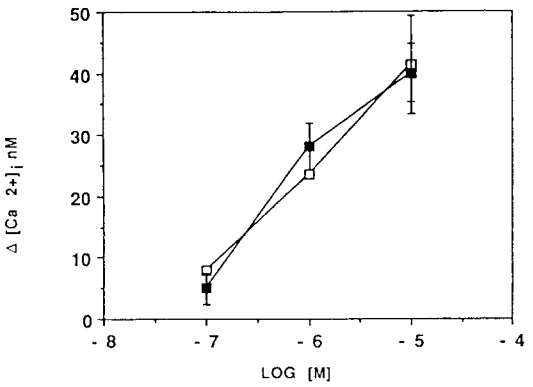

Fig. 5. Effect of PGD_2 on the $[Ca^{2+}]_i$ in the presence of (■) and absence (□) of BW A868C. Points represent mean values ± SEM, N = 3.

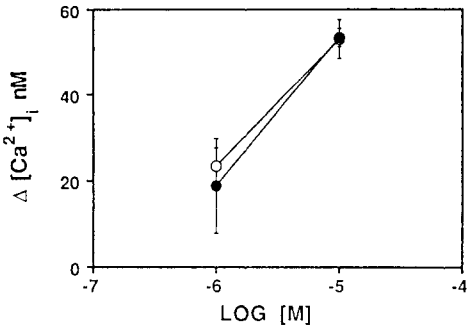

Fig. 6. Effect of U-46619 on the $[Ca^{2+}]_i$ in the presence of (●) and absence (○) of BM 13505. Points represent mean values ± SEM, N = 3.

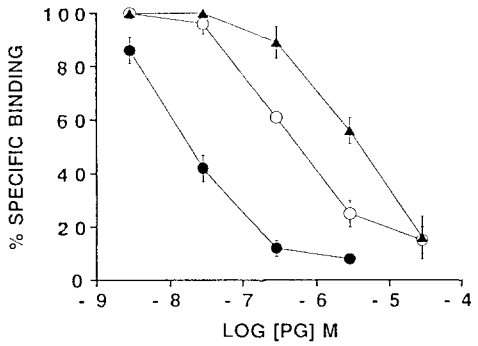

Fig. 7. Inhibition of specific $[^3H]$-$PGF_{2\alpha}$ (5×10^{-9} M) binding by $PGF_{2\alpha}$ (●), PGD_2 (○), and PGE_2 (▲). Points represent mean values ± SEM, N = 3.

receptor hypothesis is supported by studies indicating that the Ca^{2+} signal elicited by PDGF is unaffected by pretreatment with $PGF_{2\alpha}$, PGD_2 or PGE_2 despite the observation that the Ca^{2+} transient response to PDGF originates from a predominantly intracellular pool. This indirect experimental approach has been employed previously to discriminate between $PGF_{2\alpha}$ and PGE_2 responses in osteoblast-like cells, where independent receptor populations were proposed based on successive addition studies where responses to PGE_2 were not affected by $PGF_{2\alpha}$ pretreatment and vice versa [17]. These present studies in Swiss 3T3 cells resulted in an entirely different outcome, but caution is required in postulating a single receptor hypothesis based only on Ca^{2+} data. If $PGF_{2\alpha}$, PGD_2, and PGE_2 interacted with different receptor populations coupled to a single pool of intracellular Ca^{2+}, an identical experimental outcome would ensue. Thus, pretreatment with a high concentration of any prostaglandin would deplete the intracellular Ca^{2+} pool, and no response would be obtained when other receptors coupled to this pool were activated. In the present studies, results from the radioligand binding experiments are provided to support the hypothesis that a single population of receptors is involved in mediating the Ca^{2+} signal to $PGF_{2\alpha}$, PGD_2, and PGE_2.

The concept that PGD_2 stimulates the FP-receptor to elicit a Ca^{2+} signal is also supported by studies with the DP-antagonist BW A868C [5]. The Ca^{2+} transient response to PGD_2 was unaffected by BW A868C pretreatment. Moreover, the DP-receptor agonist BW 245C does not elicit a Ca^{2+} signal in Swiss 3T3 cells and does not displace $PGF_{2\alpha}$ from its binding site. These studies provide substantial evidence that the DP-receptor is not involved in mediating the Ca^{2+} response to PGD_2 in Swiss 3T3 cells. U-46619 also appears to stimulate the FP-receptor in Swiss 3T3 cells since the Ca^{2+} signal cannot be blocked by the TP-antagonist BM 13505.

In summary, the results obtained herein support the original contention [1, 2] that the FP-receptor has particular sensitivity to $PGF_{2\alpha}$ but will also recognize PGD_2 and PGE_2. These studies underscore the importance of performing adequate radioligand binding studies to identify the prostanoid receptors involved in second messenger and functional assays, despite the technical difficulties that may be associated with certain prostaglandins [28].

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