Journal of Cellular Biochemistry 54:487–493 (1994)

Effect of Prostaglandin $F_{2\alpha}$ on Ca^{2+} Influx in Osteoblast-Like Cells: Function of Tyrosine Kinase

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Abstract We previously reported that pertussis toxin–sensitive GTP-binding protein is involved in prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced phosphoinositide (PI) hydrolysis in osteoblast-like MC3T3-E1 cells [Miwa et al. (1990): Biochem Biophys Res Commun 171:1229–1235]. In the present study, we investigated the mechanism of $PGF_{2\alpha}$-induced Ca^{2+} influx in MC3T3-E1 cells. $PGF_{2\alpha}$-induced formation of total inositol phosphates (IPs) was markedly reduced by the depletion of extracellular Ca^{2+} with EGTA. On the other hand, the depletion of extracellular Ca^{2+} had little effect on $PGF_{2\alpha}$-induced inositol 1,4,5-trisphosphate formation. $PGF_{2\alpha}$ stimulated $^{45}Ca^{2+}$ influx dose dependently, attaining a maximum effect at 10 nM. Dose of $PGF_{2\alpha}$ above 10 nM caused less than maximal stimulation. Genistein, an inhibitor of protein tyrosine kinase, which by itself had little effect on $^{45}Ca^{2+}$ influx, significantly suppressed the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in a dose-dependent manner in the range between 1 μg/ml and 0.1 mg/ml. Sodium orthovanadate, an inhibitor of protein tyrosine phosphatases, enhanced the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx. Genistein also suppressed the $PGF_{2\alpha}$-induced total IPs formation dose dependently in the range between 1 μg/ml and 0.1 mg/ml. However, it had little effect on the $PGF_{2\alpha}$-induced inositol 1,4,5-trisphosphate formation. The pretreatment with pertussis toxin had little effect on the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx. These results strongly suggest that $PGF_{2\alpha}$ stimulates Ca^{2+} mobilization from extracellular space and PI hydrolysis via independent pathways in osteoblast-like cells, and the $PGF_{2\alpha}$-induced Ca^{2+} influx is regulated by protein tyrosine kinase, resulting in the promotion of PI hydrolysis.

Key words: prostaglandin $F_{2\alpha}$, calcium influx, tyrosine kinase, phosphoinositide, osteoblast

Prostaglandins are generally recognized to act as autacoids in bone metabolism [Nijweide et al., 1986; Smith, 1989]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been reported to stimulate DNA synthesis of osteoblasts and to decrease alkaline phosphatase activity, a marker of mature osteoblast phenotype [Peck et al., 1964; Stein et al., 1990], in osteoblasts [Nijweide et al., 1986; Hakeda et al., 1987; Koshihara and Kawamura, 1989], including MC3T3-E1 cells, which have been derived from newborn mouse calvaria [Kodama et al., 1981; Sudo et al., 1983]. It has also been shown that $PGF_{2\alpha}$ stimulates the proliferation of osteoblast-like MC3T3-E1 cells through phosphoinositide (PI) hydrolysis [Hakeda et al., 1987]. It is well established that PI hydrolysis by phospholipase C (PLC) results in the formation of inositol phosphates (IPs) and diacylglycerol [Berridge and Irvine, 1989]. Among these products, inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol serve as second messengers for the mobilization of Ca^{2+} from intracellular Ca^{2+} stores and the activation of protein kinase C (PKC), respectively [Berridge and Irvine, 1989; Nishizuka, 1992]. We have demonstrated that PKC is involved in the process which directs osteoblast-like MC3T3-E1 cells toward proliferation [Kozawa et al., 1989]. In a previous study [Miwa et al., 1990], we have shown that $PGF_{2\alpha}$ stimulates PI hydrolysis via pertussis toxin (PTX)-sensitive GTP-binding protein (G-protein) in these cells. As for the Ca^{2+} mobilizing system, it is well known that the intracellular Ca^{2+} level is regulated by Ca^{2+} influx from extracellular space as well as from intracellular Ca^{2+} stores [Berridge and Irvine, 1989]. However, the precise regulatory mechanism of $PGF_{2\alpha}$-induced Ca^{2+} mobilization in osteoblasts has not yet been fully clarified.

Protein tyrosine kinases are well known to be associated with the regulation of cellular prolif-

Received November 1, 1993; accepted December 14, 1993.

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eration and malignant transformation, and are classically classified with those associated with growth factor receptors and those whose genes have become part of a retrovirus, exemplified by the Src subfamily [Hunter and Cooper, 1985; Hanks et al., 1988]. It has recently been shown that several growth factors such as platelet-derived growth factor and epidermal growth factor stimulate PLC_γ, resulting in the formation of IPs [Meldrum et al., 1991]. Furthermore, it has been demonstrated that bombesin, vasopressin, and endothelin induce protein tyrosine phosphorylation as well as G-protein–coupled receptor activation of PLC in glomerular mesangial cells and Swiss 3T3 fibroblasts, and this may be the additional signal transduction pathway in mitogenesis [Force et al., 1991; Zachary et al., 1991]. In osteoblast-like MC3T3-E1 cells, $PGF_{2\alpha}$ has recently been reported to induce protein tyrosine phosphorylation [Quarles et al., 1993]. However, the details of the precise role of tyrosine kinase in osteoblasts have not yet been clarified.

In the present study, we examined the effect of $PGF_{2\alpha}$ on Ca^{2+} influx, and the effect of tyrosine kinase on the $PGF_{2\alpha}$-induced Ca^{2+} influx in osteoblast-like MC3T3-E1 cells. Herein, we show that $PGF_{2\alpha}$ separately stimulates the Ca^{2+} influx from extracellular space and PI hydrolysis in these cells, and the $PGF_{2\alpha}$-induced Ca^{2+} influx is regulated by protein tyrosine kinase, resulting in the promotion of PI hydrolysis.

MATERIALS AND METHODS

Materials

myo-[2-^{3}H]Inositol (81.5 Ci/mmol), $^{45}CaCl_2$ (10–40 mCi/mg), and the *D-myo*-[^{3}H]Ins(1,4,5)P_3 assay system were purchased from Amersham Japan (Tokyo, Japan). $PGF_{2\alpha}$ and sodium orthovanadate (vanadate) were purchased from Sigma Chemical Co. (St. Louis, MO). Genistein and PTX were purchased from Funakoshi Pharmaceutical Co. (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. $PGF_{2\alpha}$ was dissolved in ethanol. Genistein was dissolved in dimethyl sulfoxide. The maximum concentration of ethanol or dimethyl sulfoxide in the culture medium was 0.1%, and this affected neither the assay for $^{45}Ca^{2+}$ influx nor the measurement of the formation of total IPs and Ins(1,4,5)P_3.

Cell Culture

Cloned osteoblast-like MC3T3-E1 cells were generously provided by Dr. M. Kumegawa (Meikai University, Sakado, Japan) and maintained in α-minimum essential medium (α-MEM) containing 10% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 5% CO_2/95% air. The cells (5×10^4) were seeded into 35-mm-diameter dishes in 2 ml of α-MEM containing 10% FCS. After 5 d, the medium was exchanged for 2 ml of α-MEM containing 0.3% FCS. The cells were used for experiments after 48 hr. In experiments for the formation of total IPs, the medium was exchanged for 2 ml of inositol-free α-MEM containing 0.3% FCS. When indicated, the cells were pretreated with various doses of PTX for 24 hr.

Assay for $^{45}Ca^{2+}$ Influx

Assay for $^{45}Ca^{2+}$ influx was performed as previously described [Tokuda et al., 1992]. In brief, the cultured cells were pretreated with 0.1 μM nifedipine for 10 min in 1 ml of an assay buffer [5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 150 mM NaCl, 5 mM KCl, 5.5 mM glucose, 0.8 mM $MgSO_4$, and 1 mM $CaCl_2$] containing 0.01% bovine serum albumin (BSA). The cells were then stimulated by various doses of $PGF_{2\alpha}$ containing 5 μCi $^{45}Ca^{2+}$ at 37°C for the indicated periods. After washing four times with 1 ml of the cold assay buffer containing [ethylenebis-(oxyethylenenitrilo)]tetraacetic acid (EGTA), the reaction was immediately terminated by adding 1 ml of 0.1% sodium dodecyl sulfate. The radioactivity of the lysate was determined. When indicated, the cells were pretreated with genistein or vanadate for 20 min.

Measurement of the Formation of Total IPs

The cultured cells were labeled with *myo*-[2-^{3}H]inositol (3 μCi/dish) for 48 hr. The labeled cells were preincubated with 10 mM LiCl for 10 min in 1 ml of the assay buffer containing 0.01% BSA. The cells were then stimulated by various doses of $PGF_{2\alpha}$ at 37°C for the indicated periods. The reaction was terminated by adding 1 ml of 30% trichloroacetic acid. The acid supernatant was treated with diethyl ether to remove the acid and neutralized with 0.1 M NaOH. The supernatant was applied to a column of Dowex AG1-X8 (100–200 mesh, formate form). The radioactive IP was eluted from the column with 8 ml of 0.1 M formic acid containing 1 M ammonium formate [Berridge et al., 1983, 1984]. When indicated, extracellular Ca^{2+} was chelated with 6 mM EGTA.

Measurement of the Formation of $Ins(1,4,5)P_3$

Procedures were performed as described under "*Measurement of the Formation of Total IPs*" except for using unlabeled cells. $Ins(1,4,5)P_3$ in the supernatant was determined with the *D-myo*-[^{3}H]$Ins(1,4,5)P_3$ assay system.

Determination

The radioactivity of ^{45}Ca and ^3H samples was determined with a Beckman LS 6000IC liquid scintillation spectrometer.

Statistical Analysis

The data were analyzed by Student's t test, and $P < 0.05$ was considered significant. All data are presented as the mean ± SD of triplicate determinations.

RESULTS

Effect of Depletion of Extracellular Ca^{2+} With EGTA on the Formation of Total IPs and $Ins(1,4,5)P_3$ Induced by $PGF_{2\alpha}$ in MC3T3-E1 Cells

$PGF_{2\alpha}$ (10 μM) stimulated the formation of total IPs in MC3T3-E1 cells, as previously described [Miwa et al., 1990]. This effect of $PGF_{2\alpha}$ was significantly reduced by chelating extracellular Ca^{2+} with 6 mM EGTA (Fig. 1). However, the depletion of extracellular Ca^{2+} with EGTA had little effect on $PGF_{2\alpha}$-induced $Ins(1,4,5)P_3$ formation in these cells (Table I).

Dose-Dependent Effect of $PGF_{2\alpha}$ on $^{45}Ca^{2+}$ Influx and the Formation of Total IPs in MC3T3-E1 Cells

$PGF_{2\alpha}$ significantly stimulated $^{45}Ca^{2+}$ influx, even in the presence of 0.1 μM nifedipine, a Ca^{2+} antagonist that inhibits L-type voltage-dependent Ca^{2+} channels [Braunwald, 1982], time dependently up to 15 min in MC3T3-E1 cells (Fig. 2). $PGF_{2\alpha}$ stimulated $^{45}Ca^{2+}$ influx in a dose-dependent manner, attaining a maximum at 10 nM. Dose of $PGF_{2\alpha}$ above 10 nM caused less than maximal stimulation (Fig. 3). On the other hand, $PGF_{2\alpha}$ stimulated the formation of total IPs dose dependently in the range between 1 nM and 10 μM (Fig. 3). The maximum effect of $PGF_{2\alpha}$ on the total IPs formation was observed at 10 μM as previously described [Miwa et al., 1990].

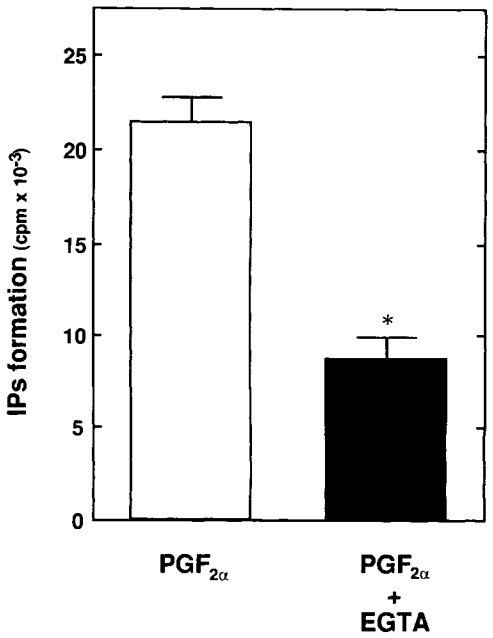

Fig. 1. Effect of depletion of extracellular Ca^{2+} on $PGF_{2\alpha}$-induced IPs formation in MC3T3-E1 cells. The [^{3}H]inositol-labeled cells were stimulated by 10 μM $PGF_{2\alpha}$ in the assay buffer with or without 6 mM EGTA for 10 min. Values for unstimulated cells have been subtracted from each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ compared to the value of $PGF_{2\alpha}$ alone.

Table I. Effect of Depletion of Extracellular Ca^{2+} on $PGF_{2\alpha}$-Induced $Ins(1,4,5)P_3$ Formation in MC3T3-E1 Cells*

EGTA	$PGF_{2\alpha}$	Ins (1,4,5)P_3 (pmol/dish)
−	−	3.8 ± 0.3
−	+	76.8 ± 3.5
+	−	3.9 ± 0.5
+	+	77.5 ± 4.0

*The cultured cells were stimulated by 10 μM $PGF_{2\alpha}$ in the assay buffer with or without 6 mM EGTA for 1 min. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Effect of Genistein on $PGF_{2\alpha}$-Induced $^{45}Ca^{2+}$ Influx in MC3T3-E1 Cells

Pretreatment with genistein, an inhibitor of protein tyrosine kinase [Akiyama et al., 1987], which by itself had little effect on $^{45}Ca^{2+}$ influx (data not shown), significantly suppressed the $PGF_{2\alpha}$ (10 nM)-induced $^{45}Ca^{2+}$ influx in a dose-dependent manner in the range between 1 μg/ml and 0.1 mg/ml in MC3T3-E1 cells (Fig. 4).

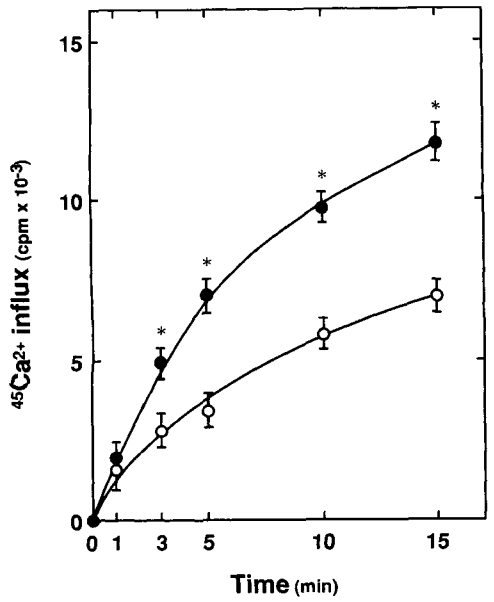

Fig. 2. Time-dependent effect of $PGF_{2\alpha}$ on $^{45}Ca^{2+}$ influx in MC3T3-E1 cells. The cultured cells were stimulated by 10 nM $PGF_{2\alpha}$ (●) or vehicle (○) with 5 μCi $^{45}Ca^{2+}$ for the indicated periods. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ compared to the value of control.

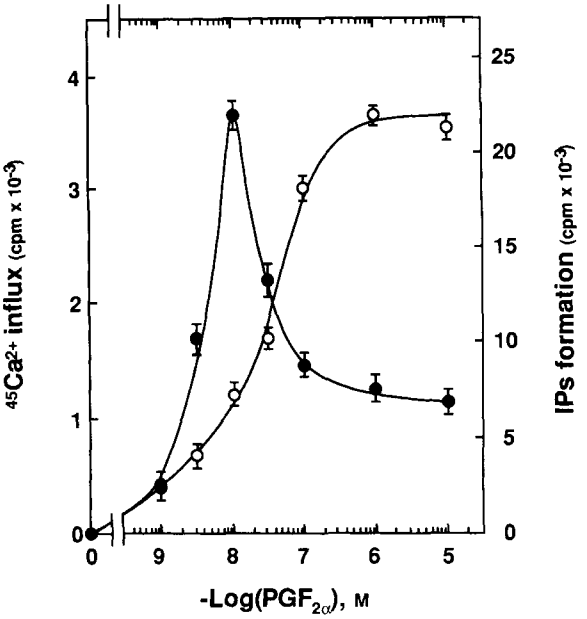

Fig. 3. Dose-dependent effect of $PGF_{2\alpha}$ on $^{45}Ca^{2+}$ influx and IPs formation in MC3T3-E1 cells. The cultured cells were stimulated by various doses of $PGF_{2\alpha}$ with 5 μCi $^{45}Ca^{2+}$ for 5 min, and the $^{45}Ca^{2+}$ influx (●) was then determined. As for the measurement of IPs, the [^{3}H]inositol-labeled cells were stimulated by various doses of $PGF_{2\alpha}$ for 10 min, and the formation of IPs (○) was then determined. Values for unstimulated cells have been subtracted from each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Effect of Vanadate on $PGF_{2\alpha}$-Induced $^{45}Ca^{2+}$ Influx in MC3T3-E1 Cells

To clarify the role of tyrosine kinase in $PGF_{2\alpha}$-induced Ca^{2+} influx, we examined the effect of vanadate, an inhibitor of protein tyrosine phosphatases [Klarlund et al., 1988], on the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in MC3T3-E1 cells. The pretreatment with 0.1 mM vanadate, which by itself had little effect on $^{45}Ca^{2+}$ influx, significantly enhanced the $PGF_{2\alpha}$ (3 nM)-induced $^{45}Ca^{2+}$ influx in these cells (Fig. 5).

Effect of Genistein on the Formation of Total IPs and $Ins(1,4,5)P_3$ by $PGF_{2\alpha}$ in MC3T3-E1 Cells

We next examined the effect of genistein on the $PGF_{2\alpha}$-induced PI hydrolysis in MC3T3-E1 cells. The pretreatment with genistein, which by itself had little effect on total IPs formation (data not shown), markedly suppressed the $PGF_{2\alpha}$ (10 μM)-induced total IPs formation in a dose-dependent manner in the range between 1 μg/ml and 0.1 mg/ml in MC3T3-E1 cells (Fig. 6). On the other hand, the pretreatment with 30 μg/ml of genistein, which is a submaximum dose for the inhibitory effect on $PGF_{2\alpha}$-induced total IPs formation, had little effect on the $PGF_{2\alpha}$ (10 μM)-induced $Ins(1,4,5)P_3$ formation in these cells (Table II).

Effect of PTX on $PGF_{2\alpha}$-Induced $^{45}Ca^{2+}$ Influx in MC3T3-E1 Cells

We previously showed that $PGF_{2\alpha}$ stimulates PI hydrolysis via PTX-sensitive G-protein in MC3T3-E1 cells [Miwa et al., 1990]. We examined the effect of PTX on $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in these cells. The pretreatment with PTX in the range between 1 ng/ml and 1 μg/ml had little effect on the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in MC3T3-E1 cells (Fig. 7).

DISCUSSION

In the present study, we demonstrated that the depletion of extracellular Ca^{2+} with EGTA significantly reduced the $PGF_{2\alpha}$-induced formation of total IPs in osteoblast-like MC3T3-E1 cells. On the contrary, the depletion of extracellular Ca^{2+} had little effect on $PGF_{2\alpha}$-induced $Ins(1,4,5)P_3$ in these cells. It has been shown that $Ins(1,4,5)P_3$ formation from phosphatidylinositol-4,5-bisphosphate by PLC is largely inde-

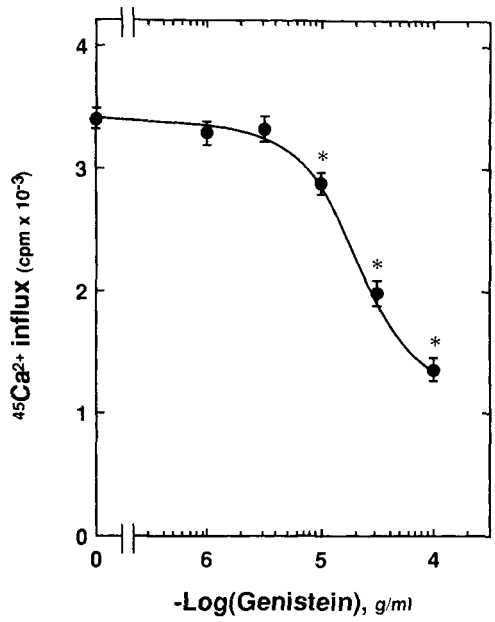

Fig. 4. Effect of genistein on $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in MC3T3-E1 cells. The cultured cells were pretreated with various doses of genistein for 20 min, then stimulated by 10 nM $PGF_{2\alpha}$ with 5 μCi $^{45}Ca^{2+}$ for 5 min. Values for unstimulated cells have been subtracted from each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ compared to the value without genistein pretreatment.

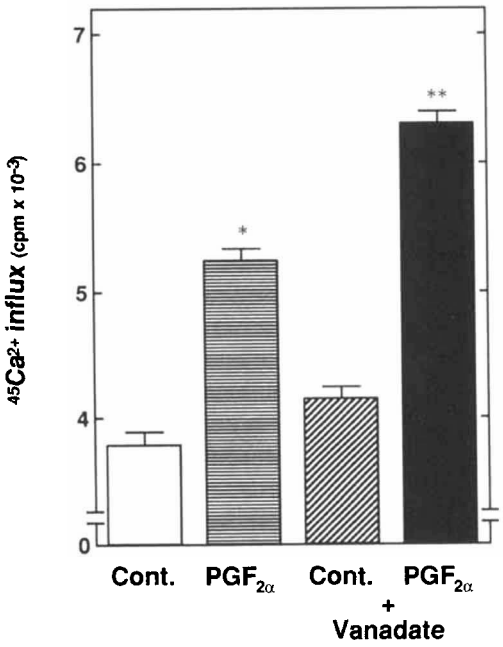

Fig. 5. Effect of vanadate on $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in MC3T3-E1 cells. The cultured cells were pretreated with 0.1 mM vanadate or vehicle for 20 min, then stimulated by 3 nM $PGF_{2\alpha}$ or vehicle with 5 μCi $^{45}Ca^{2+}$ for 5 min. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ compared to the value of control. **$P < 0.05$ compared to the value of $PGF_{2\alpha}$ alone.

pendent of the Ca^{2+} level [Meldrum et al., 1991]. It has also been demonstrated that the Ins(1,4,5)P_3 formation precedes both the formation of inositol-1-phosphate from phosphatidylinositol and the formation of inositol-1,4-bisphosphate from phosphatidylinositol-4-phosphate [Meldrum et al., 1991]. It is well recognized that the mobilization of intracellular Ca^{2+} increases the rate of PI hydrolysis [Fain, 1990]. In the present study, we confirmed that $PGF_{2\alpha}$-induced formation of total IPs depends on the Ca^{2+} influx from extracellular space, while the Ca^{2+} influx has little effect on the $PGF_{2\alpha}$-induced Ins(1,4,5)P_3 formation in MC3T3-E1 cells.

To clarify the mechanism of $PGF_{2\alpha}$-induced Ca^{2+} influx, we next compared the dose dependency of $PGF_{2\alpha}$ on Ca^{2+} influx with that on total IPs formation in these cells. We demonstrated that $PGF_{2\alpha}$ stimulated Ca^{2+} influx in a dose-dependent manner, attaining a maximum at 10 nM, and a dose of $PGF_{2\alpha}$ above 10 nM caused less than maximal stimulation. On the other hand, $PGF_{2\alpha}$ stimulated the formation of total IPs dose dependently in the range between 1 nM and 10 μM. The maximum effect of $PGF_{2\alpha}$ was 10 μM. So, from our findings that these dose-dependent curves were quite different, it seems unlikely that $PGF_{2\alpha}$ stimulates PI hydrolysis and Ca^{2+} influx by a common pathway. It has been reported that Ins(1,4,5)P_3 and/or inositol 1,3,4,5-tetrakisphosphate, a metabolite from Ins(1,4,5)P_3 by Ins(1,4,5)P_3-3-kinase, mobilize Ca^{2+} also from extracellular space [Berridge and Irvine, 1989]. In a previous study [Miwa et al., 1990], we have shown that $PGF_{2\alpha}$ stimulates PI hydrolysis in a PTX-sensitive manner, resulting in the formation of IPs in MC3T3-E1 cells. We showed here that the pretreatment with PTX had little effect on $PGF_{2\alpha}$-induced Ca^{2+} influx in these cells. Thus, it seems unlikely that PTX-sensitive G-protein is involved in the $PGF_{2\alpha}$-induced Ca^{2+} influx and that $PGF_{2\alpha}$ stimulates Ca^{2+} influx from extracellular space due to the production of Ins(1,4,5)P_3 and/or inositol 1,3,4,5-tetrakisphosphate by PI hydrolysis. These results strongly suggest that $PGF_{2\alpha}$ separately stimulates Ca^{2+} influx and PI hydrolysis in osteoblast-like MC3T3-E1 cells.

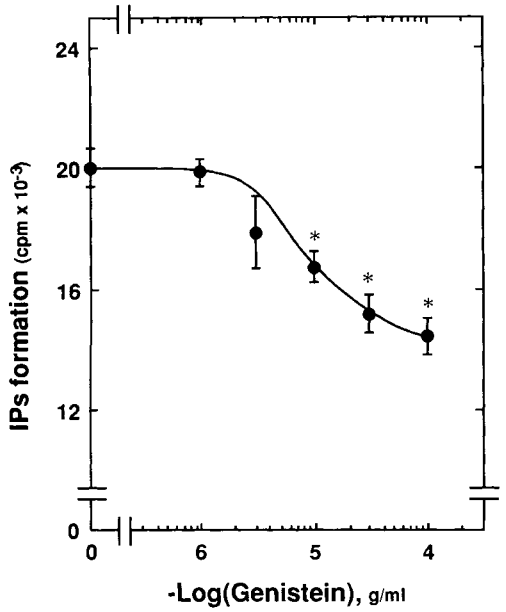

Fig. 6. Effect of genistein on $PGF_{2\alpha}$-induced IPs formation in MC3T3-E1 cells. The [^{3}H]inositol-labeled cells were pretreated with various doses of genistein for 20 min, then stimulated by 10 μM $PGF_{2\alpha}$ for 10 min. Values for unstimulated cells have been subtracted from each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ compared to the value without genistein pretreatment.

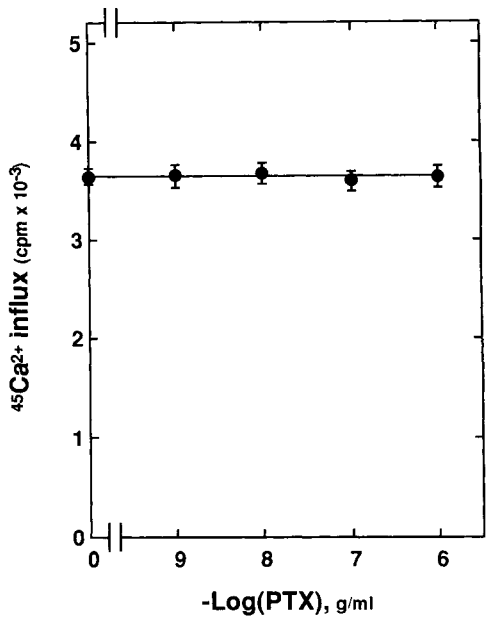

Fig. 7. Effect of PTX on $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in MC3T3-E1 cells. The cultured cells were pretreated with various doses of PTX for 24 hr, and then stimulated by 10 nM $PGF_{2\alpha}$ with 5 μCi $^{45}Ca^{2+}$ for 5 min. Values for unstimulated cells have been subtracted from each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

TABLE II. Effect of Genistein on $PGF_{2\alpha}$-Induced Ins(1,4,5)P_3 Formation in MC3T3-E1 Cells*

Genistein	$PGF_{2\alpha}$	Ins (1,4,5)P_3 (pmol/dish)
−	−	4.0 ± 0.5
−	+	85.6 ± 9.5
+	−	4.2 ± 0.8
+	+	78.0 ± 5.0

*The cultured cells were penetrated with 30 μg/ml genistein for 20 min, then stimulated by 10 μM $PGF_{2\alpha}$ for 1 min. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

It has recently been reported that $PGF_{2\alpha}$ induces tyrosine phosphorylation in MC3T3-E1 cells [Quarles et al., 1993]. Accumulating evidence suggests that tyrosine kinase is activated by agonists such as vasopressin and endothelin acting through receptors linked to G-protein, and this may be the additional signal transduction pathway in mitogenesis [Force et al., 1991; Zachary et al., 1991]. We previously demonstrated that PTX-sensitive G-protein is involved in the coupling of $PGF_{2\alpha}$ receptor to PLC in MC3T3-E1 cells [Miwa et al., 1990]. In the present study, we showed that the pretreatment with genistein, known as an inhibitor of protein tyrosine kinase [Akiyama et al., 1987], significantly inhibited the $PGF_{2\alpha}$-induced Ca^{2+} influx in these cells. In addition, we also showed that vanadate, an inhibitor of protein tyrosine phosphatases [Klarlund et al., 1988], enhanced the $PGF_{2\alpha}$-induced Ca^{2+} influx. These results suggest that protein tyrosine kinase is involved in the $PGF_{2\alpha}$-induced Ca^{2+} influx in osteoblast-like MC3T3-E1 cells. We next demonstrated that the pretreatment with genistein inhibited the $PGF_{2\alpha}$-induced total IPs formation in these cells. On the other hand, genistein had little effect on the formation of Ins(1,4,5)P_3. It has been shown that PLC_γ is activated by several tyrosine kinase receptor systems in a manner which is apparently independent of G-protein [Meldrum et al., 1991]. However, it seems unlikely that $PGF_{2\alpha}$-induced protein tyrosine kinase directly stimulates PLC in MC3T3-E1 cells. From our findings as a whole, it is most likely that $PGF_{2\alpha}$ activates both PTX-sensitive G-protein–coupled PLC and protein tyrosine kinase, resulting in Ins(1,4,5)P_3 formation and Ca^{2+} influx, respectively, and sub-

sequently the Ca^{2+} influx promotes the PI hydrolysis by $PGF_{2\alpha}$ in MC3T3-E1 cells.

It is well accepted that the activation of protein tyrosine kinase and the activation of PKC have mitogenic and proliferative effects on ubiquitous cells [Hunter and Cooper, 1985; Nishizuka, 1992]. It has been reported that $PGF_{2\alpha}$ stimulates the proliferation of osteoblast-like MC3T3-E1 cells through PI hydrolysis [Hakeda et al., 1987]. We previously reported that PKC, which is physiologically activated by diacylglycerol produced from PI hydrolysis, is involved in the process which directs MC3T3-E1 cells toward proliferation [Kozawa et al., 1989]. These results suggest that $PGF_{2\alpha}$-induced protein tyrosine kinase plays an important role in the promotion of $PGF_{2\alpha}$-induced PI hydrolysis, resulting in the proliferation of osteoblast-like MC3T3-E1 cells.

In conclusion, our results strongly suggest that $PGF_{2\alpha}$ stimulates the Ca^{2+} mobilization from extracellular space and PI hydrolysis via independent pathways in osteoblast-like cells, and the $PGF_{2\alpha}$-induced Ca^{2+} influx is regulated by tyrosine kinase, resulting in the promotion of PI hydrolysis.

ACKNOWLEDGMENTS

This investigation was supported in part by a Grant-in-Aid for scientific research from the Ministry of Education, Science, and Culture of Japan.

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