Intracellular mechanisms underlying prostaglandin \( F_{2\alpha} \)-stimulated phasic myometrial contractions

MARK PHILLIPPE, TREVANIA SAUNDERS, AND ANDREW BASA
Department of Obstetrics and Gynecology, University of Chicago, Chicago, Illinois 60637

Intracellular mechanisms underlying prostaglandin \( F_{2\alpha} \)-stimulated phasic myometrial contractions. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E665–E673, 1997.—These studies sought to test the hypothesis that prostaglandin \( F_{2\alpha} \) (PGF\(_{2\alpha}\))-stimulated phasic myometrial contractions are characterized by the activation of the phosphatidylinositol-signaling pathway resulting in the generation of cytosolic calcium oscillations. For the experiments described in this report rat myometrial tissue was used, after the tissue was loaded with fura 2, to perform cytosolic calcium imaging studies and to perform computer-digitalized in vitro isometric contraction studies. Consistent with the above hypothesis, the cytosolic calcium imaging studies demonstrated PGF\(_{2\alpha}\)-stimulated cytosolic calcium oscillations occurring simultaneously with phasic contractions. The in vitro isometric contraction studies confirmed that previously reported inhibitors of the phosphatidylinositol-sensing pathway and cytosolic calcium oscillation mechanisms resulted in significant inhibition of PGF\(_{2\alpha}\)-stimulated phasic myometrial contractions. In summary, these studies have provided substantial support for the hypothesis that PGF\(_{2\alpha}\)-stimulated phasic myometrial contractions are generated by intracellular signaling mechanisms involving activation of the phosphatidylinositol-signaling pathway and the production of cytosolic calcium oscillation-like phenomena.

CYTOSOLIC CALCIUM oscillations have been previously reported to occur in many types of excitable and nonexcitable cells in response to stimulation by various hormones and neurotransmitters; these reports have also confirmed that the phosphatidylinositol (PI)-signaling pathway plays a significant role during the generation of these calcium oscillations (2, 30). During these phenomena, receptor-activated stimulation of phosphoinositide-specific phospholipase C (PI-PLC) has been shown to result in increased production of inositol phosphates, especially inositol 1,4,5-trisphosphate (IP\(_3\)), which leads to the release of sequestered intracellular calcium. Subsequently, cycles of calcium release and reuptake by the intracellular stores appear to result in sustained oscillations of cytosolic calcium. Additional biochemical events that have been reported to contribute to these phenomena include activation of calcium-induced calcium release (CICR) from IP\(_3\)-insensitive calcium stores, influx of extracellular calcium through membrane calcium channels, negative feedback by protein kinase C, and possible oscillations in IP\(_3\) production (2, 30).

Several recent reports have demonstrated that phasic smooth muscle contractions, especially those occurring in female genital tract smooth muscle, occur simultaneously with repetitive transients (or oscillations) of cytosolic calcium (7, 8, 19, 23, 24, 28). These smooth muscle calcium oscillations appear to be similar, if not identical, to the cytosolic calcium oscillations observed in excitable and nonexcitable cells as described above. Previous reports from our laboratory have confirmed that activation of the PI-signaling pathway using various agonists, including oxytocin, aluminum fluoride (a G protein activator), potassium chloride (at concentrations of 10–30 mM), ionomycin, and BAY K 8644, results in the generation of phasic myometrial contractions (21–24). Consistent with classic cytosolic calcium oscillation phenomena, agonist-stimulated phasic myometrial contractions were found to be markedly suppressed by 1) enzyme inhibitors of PI-PLC, 2) membrane permeant inhibitors of intracellular calcium uptake and release, 3) phorbol ester activation of protein kinase C, and 4) inhibition of extracellular calcium influx (21–24).

Until recently, the signal transduction mechanisms underlying prostaglandin stimulation have been both unclear and controversial. Several reports have confirmed that prostanoids bind to cell surface membrane receptors; to date, one prostaglandin F (PGF) membrane receptor and four prostaglandin E (PGE) membrane receptors have been cloned and sequenced (18, 27). The PGE receptors appear to use multiple intracellular signaling pathways (18), whereas the PGF receptor appears to predominantly activate the PI-signaling pathway (27). Although previous reports have confirmed inositol phosphate production in response to PGF\(_{2\alpha}\) stimulation of uterine myometrial cells (5, 13, 15, 20), the role of this signaling pathway during PGF\(_{2\alpha}\)-stimulated phasic myometrial contractions has not been clearly defined. The studies described in this report were undertaken to test the hypothesis that PGF\(_{2\alpha}\)-stimulated phasic myometrial contractions are generated by intracellular signaling mechanisms involving activation of the PI-signaling pathway and the production of cytosolic calcium oscillation-like phenomena.

MATERIALS AND METHODS

For these experiments, myometrial tissue was obtained from mature, proestrus/estrus Sprague-Dawley white rats, using a protocol approved by the Institutional Animal Care and Utilization Committee of the University of Chicago. Under pentobarbital sodium (100 mg/kg) anesthesia, the uteri were surgically removed, rinsed in normal saline, then placed in Earle’s buffered salt solution [EBSS; (in mM) 117 NaCl, 1.8 CaCl\(_2\), 5.3 KCl, 0.8 MgSO\(_4\), 1 NaH\(_2\)PO\(_4\), 26.2 NaHCO\(_3\), and 5.6 glucose, pH 7.4] continuously bubbled with 95% O\(_2\)-5% CO\(_2\).

The cytosolic calcium imaging studies were performed using the longitudinal fiber layer of the myometrium, as previously reported (23, 24). Specifically, partial thickness contraction studies confirmed that previously reported inhibitory mechanisms involving activation of the phosphatidylinositol-sensing pathway and cytosolic calcium oscillation mechanisms resulted in significant inhibition of PGF\(_{2\alpha}\)-stimulated phasic myometrial contractions. In summary, these studies have provided substantial support for the hypothesis that PGF\(_{2\alpha}\)-stimulated phasic myometrial contractions are generated by intracellular signaling mechanisms involving activation of the phosphatidylinositol-sensing pathway and the production of cytosolic calcium oscillation-like phenomena.
myometrial strips (~4 × 6 mm) were prepared with the use of a dissecting microscope while the tissue was kept in freshly aerated EBSS. Subsequently, the strips were rinsed in a N-2-hydroxyethylpiperazine-N',N'-2-ethanesulfonic acid (HEPES)-buffered physiological salt solution [(in mM) 150 NaCl, 2 CaCl₂, 4 KCl, 1 MgCl₂, 5.6 glucose, and 5 HEPES, pH 7.4] aerated with O₂ and then loaded with fura 2, a ratiometric fluorescent intracellular calcium-indicator dye. The loading incubations in freshly oxygenated HEPES-buffered salt solution containing 2.5 µM fura 2-AM (the membrane-permeant ester form of fura 2) along with 0.008% pluronic acid were performed for 18–22 h at room temperature. Subsequently, the myometrial tissue was pinned to parallel rubber strips attached to the bottom of a heated perfusion dish (Delta-T Culture Dish System, Biotech, Butler, PA) mounted on a Nikon TMS inverted microscope modified for epifluorescence imaging.

To simultaneously determine force generation, the myometrial strips were attached to a horizontal isometric force transducer (Kent Scientific, Litchfield, CT) using stainless steel hooks. Before the initiation of the experiments, the tissue was perfused in the dish with fresh buffer at room temperature for at least 20–30 min to facilitate completion of the hydrolysis of the ester form of the fura 2. During the subsequent calcium imaging experiments, the tissue was continuously perfused with oxygenated buffer at 30°C. The low temperature was used to decrease the rate of extrusion of the fluorescent indicator dye; at this temperature spontaneous activity of the muscle was uncommon. Tissue perfusion studies were performed using 10 nM or 0.5 µM PGF₂α with and without various inhibitors, including 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC) and neomycin [reported inhibitors of PI-PLC (9, 11, 14, 21)], thimerosal [a biphasic modulator of IP₃-sensitive calcium release and uptake (4, 26)], adenine and rubidium red [reported inhibitors of CICR (6, 22, 25)], and nifedipine [an L-type calcium channel blocker (29)]. The fura 2 cytosolic calcium measurements were performed using a video-based digital calcium imaging system (Intracellular Calcium Imaging, Cincinnati, OH) setup for dual excitation at 340 and 380 nm and emission at 505 nm. The calcium results were reported as the relative fluorescence intensities of the 340 and 380 images and as the 340-to-380 ratio (340/380). The calcium transients stimulated in response to oxytocin were compared with those produced in response to a brief pulse of KCl (50 mM).

In vitro isometric contraction studies were performed at 37°C using longitudinal strips of uterine tissue (6–8 mm relaxed length) in 50-ml muscle baths, as previously reported from our laboratory (21, 22). Individual strips from individual animals were used for each of the studies described in this
report. Cumulative dose-response studies were performed using PGF$_{2\alpha}$ (1 nM to 1 µM) with and without NCDC (80–120 µM), neomycin (1–5 mM), thimerosal (100–500 µM), adenine (1–5 mM), and nifedipine (1 µM). The effects of these inhibitors were compared with control studies performed using comparable volumes of vehicle alone. For the vehicle control studies performed using ethanol or dimethyl sulfoxide (DMSO), the maximal concentration of ethanol in the muscle bath was 0.2 µl/ml, and the maximal concentration of DMSO was 2.4 µl/ml. To further evaluate the effects of extracellular calcium on PGF$_{2\alpha}$-stimulated phasic contractions, additional studies were performed during which the EBSS buffer was rapidly replaced with preoxygenated, prewarmed calcium-free EBSS buffer containing 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA).

The analog data generated by the isometric contraction transducers were digitalized and then analyzed to determine total contractile activity (based on the area under the contraction curve) for 5-min intervals, as previously reported from our laboratory (21, 22). Subsequently, the contraction data were normalized for tissue cross-section area and reported as the percentage of spontaneous or PGF$_{2\alpha}$-stimulated contractile activity. All studies were performed using 4–12 replicates, as noted. Statistical analysis was performed using Kruskal-Wallis one-way analysis of variance on ranks, the multiple comparisons Student-Newman-Keuls and Dunn tests, and the Mann-Whitney rank sum test where appropriate (significance = P < 0.05).

RESULTS

The in vitro isometric contraction studies confirmed concentration-related stimulation of an increase of phasic contractile activity, consisting of an increase in both frequency and intensity, in response to PGF$_{2\alpha}$ (Fig. 1A). As demonstrated in Fig. 1B, the increase in contractile activity was apparent with 10 nM PGF$_{2\alpha}$ and statistically significant at 100 nM and 1 µM compared with strips treated with vehicle alone (P < 0.05). A representative recording of simultaneous cytosolic calcium transients and isometric contractile activity is demonstrated in Fig. 2. As observed, PGF$_{2\alpha}$ stimulated a small increase in basal cytosolic calcium along with the development of calcium oscillations. The
Cytosolic calcium oscillations were associated with simultaneous phasic myometrial contractions. Of note, the relative magnitude of these PGF2α-stimulated calcium oscillations and contractions were comparable to the calcium transient and tonic contraction produced by the brief pulse with 50 mM KCl.

NCDC, a membrane-permeant inhibitor of phospholipase C, markedly suppressed the frequency and intensity of PGF2α-stimulated in vitro isometric contractions. As demonstrated in Fig. 3A, the NCDC effect was reversed after washout and restimulation. This concentration-related NCDC effect became significant at concentrations of 80 µM and greater, compared with uterine strips treated with comparable volumes of vehicle (DMSO) alone (P < 0.05, Fig. 3B). As observed in Fig. 4, NCDC completely inhibited the cytosolic calcium oscillations and associated contractions produced by 10 nM PGF2α (Fig. 5). As observed in Fig. 6, neomycin, another inhibitor of phospholipase C, produced significant inhibition of PGF2α-stimulated phasic myometrial contractions at concentrations of 1 mM and greater (P < 0.05). Comparable to NCDC, the effect of neomycin was reversible with washout of the reagent and restimulation (Fig. 6A). Consistent with its effects on phasic contractions, neomycin produced complete inhibition of PGF2α-stimulated calcium oscillations (Fig. 7).

As discussed previously, the cycling of intracellular calcium into and out of the endoplasmic reticulum calcium stores is an important component of the intracellular mechanisms underlying classic cytosolic calcium oscillations; the same phenomena appear to be used during PGF2α-stimulated phasic myometrial contractions. Modulation of IP3-dependent calcium release by thimerosal, a membrane-permeant modulator of IP3-receptor activity and calcium uptake, resulted in a biphasic effect on PGF2α-stimulated phasic contractions. Lower concentrations of thimerosal mildly stimulated myometrial contractions; whereas, higher concentrations significantly inhibited the contractile activity (P < 0.05, Fig. 8B). As demonstrated in Fig. 8A, reversal of the effects of this sulfhydryl reagent required washout followed by treatment of the tissue.
with dithiothreitol (DTT), a thiol-reducing agent, and restimulation with PGF
\[\text{a} \]. In the absence of the DTT pretreatment, the second addition of PGF2
\[\text{a} \] was ineffective (data not shown). As observed in Fig. 8, thimerosal inhibition of phasic contractions is associated with a transient increase in basal muscle tension; the cytosolic calcium imaging studies confirmed a comparable rise in cytosolic calcium as thimerosal inhibited the calcium oscillations in myometrial tissue (Fig. 9).

As noted previously, adenine has been reported to inhibit CICR. Consistent with this reported effect, adenine reversibly inhibited PGF2\[\text{a} \] stimulated phasic contractions, as observed in Fig. 10A. At concentrations of 1 mM and greater, this adenine effect was significant compared with uterine strips treated with comparable volumes of vehicle alone (\(P < 0.05\), Fig. 10B). As demonstrated in Fig. 11, the effects of adenine on contractile force appeared greater than its effects of calcium oscillations. These observations suggest that adenine has a potent effect on the contractile apparatus in uterine myocytes, in addition to its effects of CICR. Confirming that CICR from the intracellular ryanodine receptors play an important role during these phenomena, calcium imaging studies performed using ruthenium red demonstrated suppression of the frequency and intensity of PGF2\[\text{a} \] stimulated calcium oscillations and associated contractions (Fig. 12). A previous report from our laboratory has confirmed the ability of ruthenium red to markedly inhibit in vitro myometrial contractions stimulated in response to several uterotoninc agonists, including PGF2\[\text{a} \] (22).

During classic cytosolic calcium oscillations, the influx of extracellular calcium appears to be essential for both supplementation of the cytosolic calcium transients and refill of the intracellular calcium stores. A similar need for extracellular calcium appears to occur during PGF2\[\text{a} \] stimulated phasic myometrial contractions. Consistent with this requirement for the influx of extracellular calcium, the studies performed using nifedipine, a membrane calcium channel blocker, confirmed significant inhibition of PGF2\[\text{a} \] stimulated phasic myometrial contractions (\(P < 0.05\), Table 1). Significant inhibition of PGF2\[\text{a} \] stimulated contractile activity

Fig. 7. Representative recording of cytosolic calcium transients and simultaneous contractile activity of a fura 2-loaded myometrial strip treated with 0.5 \(\mu\)M PGF2\[\text{a} \] and 2.5 mM neomycin. Top: changes in relative fluorescent intensity of intracellular fura 2 after excitation at 340 and 380 nm. Middle: relative changes in concentration of cytosolic calcium as indicated by 340/380. Bottom: isometric contractile activity in grams of tension generated in fura 2-loaded myometrial strip. Myometrial strip was treated with a brief pulse of KCl (50 mM) as indicated. Subsequently, infusion of HEPES-buffered salt solution containing PGF2\[\text{a} \] (as indicated by horizontal bar) resulted in generation of calcium oscillations and simultaneous contractions. Addition of neomycin to infusion resulted in complete inhibition of these effects.

Fig. 8. Dose-response effects of thimerosal (THIM) on PGF2\[\text{a} \] stimulated myometrial contractile activity. A: representative in vitro contraction study demonstrating reversal of THIM effect, activity in grams tension generated during spontaneous contraction period preceding 20 min, addition of PGF2\[\text{a} \] (1 \(\mu\)M), addition of THIM (500 \(\mu\)M), washout with warm saline and fresh buffer, addition of 1 mM dithiothreitol (DTT), a second washout with warm saline and fresh buffer, followed by a second addition of PGF2\[\text{a} \]. B: cumulative effects of THIM on PGF2\[\text{a} \] stimulated (1 \(\mu\)M) myometrial contractions. Each bar = mean \(\pm\) SD; \(n = 9\) experiments. Open bars, strips treated with THIM; hatched bars, strips treated with vehicle (distilled water) alone. \(\star P < 0.05\) for pretreatment control periods compared with PGF2\[\text{a} \] stimulated contractile activity; and \(* P < 0.05\) for treatment periods compared with PGF2\[\text{a} \] stimulated contractile activity and compared with vehicle controls.
was also observed in response to removal of extracellular calcium by replacement of the EBSS buffer with calcium-free EBSS buffer containing 2 mM EGTA (*P*, 0.05, Table 1).

**DISCUSSION**

The studies described in this report have provided evidence to support the hypothesis that PGF$_2\alpha$-stimulated contractions occur in response to the generation of cytosolic calcium oscillation-like phenomena. The in vitro contraction studies, which confirmed suppression of PGF$_2\alpha$-stimulated phasic myometrial contractions in response to several inhibitors of the PI-signaling pathway, are quite consistent with the previously reported effects of these reagents. Of interest, these inhibitors suppressed phasic contractile activity below baseline activity, suggesting that the spontaneous activity of myometrial smooth muscle is dependent on some level of constitutive activity of the PI-signaling pathway. The cytosolic calcium imaging studies described in this report have clearly demonstrated periodic calcium transients associated with simultaneous myometrial contractions in response to PGF$_2\alpha$; these myometrial calcium transients are consistent with cytosolic calcium oscillations observed in other cells. Similar cytosolic calcium oscillation-like phenomena have been reported in response to oxytocin and other uterotonic agonists using both cultured uterine myocytes and strips of myometrial tissue (3, 8, 12, 23, 24, 28).

The cytosolic calcium results described in our studies using fura 2, a ratiometric calcium indicator, have been reported in relative changes in cytosolic calcium based on 340/380; however, other investigators have reported the absolute concentrations of cytosolic calcium in myometrial tissue. Szal et al. (28) used aequorin (a calcium-sensitive bioluminescent protein) loaded human myometrial tissue to measure resting cytosolic calcium levels of 158 nM and peak stimulated levels of 210–390 nM. These investigators also observed that the half-maximal effective calcium concentration with respect to force generation was 172 nM in myometrial tissue. Anwer et al. (1) and Criswell et al. (3), using fura 2, a ratiometric calcium indicator, have reported similar cytosolic calcium oscillation-like phenomena in response to oxytocin and other uterotonic agonists using both cultured uterine myocytes and strips of myometrial tissue.
lar basal and stimulated cytosolic calcium concentrations in cultured uterine myocytes. Because of concerns regarding the potential difficulty in exactly reproducing the intracellular molecular conditions during the calibration of these calcium indicator dyes, many investigators are currently reporting cytosolic calcium data in relative changes based on 340/380 (e.g., Refs. 8 and 12) rather than absolute concentrations of cytosolic calcium.

Until recently, the signal transduction mechanisms mediating the intracellular effects of prostaglandins, especially PGE and PGF$_{2\alpha}$, have been unclear. Using corpus luteal cells, Leung et al. (10) demonstrated in 1986 that PGF$_{2\alpha}$ stimulated a rapid, concentration-related decrease in membrane PI mono- and bisphosphates, along with an increase in inositol polyphosphates, including IP$_3$. Similar phenomena have been reported in myometrial tissue in response to stimulation with PGs, especially PGF$_{2\alpha}$. Using cultured human myometrial cells, Molnár and Hertelendy (16) reported an increase in inositol phosphate production in response to stimulation with PGF$_{2\alpha}$.

### Table 1. Effects of extracellular calcium influx on PGF$_{2\alpha}$-stimulated phasic myometrial contractions

<table>
<thead>
<tr>
<th>Incubation Conditions</th>
<th>n</th>
<th>Percent Control Activity (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF$_{2\alpha}$ (control)</td>
<td>6</td>
<td>100.0 (0.0)</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ + 1 µM nifedipine</td>
<td>6</td>
<td>20.4* (8.9)</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ + vehicle (EtOH)</td>
<td>6</td>
<td>89.2 (5.6)</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ then EBSS-EGTA</td>
<td>4</td>
<td>9.0* (4.3)</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ then EBSS</td>
<td>4</td>
<td>64.0 (3.8)</td>
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Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) concentration was 1 µM for all of these experiments. Volume of vehicle [ethanol (EtOH)] was the same as used for the nifedipine (i.e., 10 µl in a 50-ml muscle bath). Uterine strips were stimulated with PGF$_{2\alpha}$ for 10 min, and then Earle’s buffered salt solution (EBSS; see MATERIALS AND METHODS) was rapidly replaced with preoxygenated calcium-free EBSS containing 2 mM EGTA. Uterine strips were stimulated with PGF$_{2\alpha}$ for 10 min, and then the EBSS was rapidly replaced with fresh, preoxygenated EBSS. *Significant difference (P < 0.05) compared with PGF$_{2\alpha}$ alone and to vehicle control studies.
response to 1–10 μM PGF₂α, albeit to a lesser degree than that produced by oxytocin. However, these investigators have suggested that the effects of PGF₂α, especially at low nanomolar concentrations, are dependent on the influx of extracellular calcium by mechanisms unrelated to activation of phospholipase C and the generation of IP₃ (15, 17). In contrast, other investigators have clearly observed a relationship between PGF₂α stimulation, activation of the PI-signaling pathway, and elevation of cytosolic calcium. In 1992, Goureau et al. (5) demonstrated the rapid, concentration-related stimulation of inositol phosphate production in both estrogen-dominanted and pregnant rat myometrial tissue in response to PGs; PGF₂α, was found to be the most potent of the prostanoïds tested. More recently, Phaneuf et al. (20) reported PGF₂α activation of the PI-signaling pathway in cultured human myometrial cells. In contrast to the reports by Molnár and Hertelendy (15, 17), these investigators found that the effects of PGF₂α were not dependent on the influx of extracellular calcium. Phaneuf et al. (20) have also provided evidence demonstrating that members of the Gq class of G protein α-subunits couple membrane PGF receptors to the PI-signaling pathway in uterine myocytes. Our observations that PGF₂α-stimulated calcium oscillations and phasic contractions were markedly suppressed by membrane permeant phospholipase C inhibitors provide further evidence that the PI-signaling pathway plays an important role during these phenomena. Interestingly, these inhibitor effects were apparent even at low nanomolar concentrations of PGF₂α. Addressing the facilitative role of extracellular calcium during these signaling phenomena, Maka et al. (13) observed that extracellular calcium depletion completely inhibited inositol phosphate production stimulated in response to both oxytocin and PGF₂α. Therefore, extracellular calcium appears necessary, not only for the supplementation of the cytosolic calcium transient and refill of the intracellular calcium stores, but also to facilitate the activation of agonist-stimulated phospholipase C in myometrial smooth muscle cells.

Recently the PG membrane receptors have been characterized at a molecular level. PGE receptors have been reported to activate multiple second messenger pathways, including modulation of adenylate cyclase activity, inositol phosphate production, and regulation of cytosolic calcium (18). In contrast, the PGF receptor appears to be coupled to a second messenger pathway, resulting only in the activation of the PI-signaling pathway and stimulation of increased cytosolic calcium (27). To date, four distinct PGE receptors have been cloned and sequenced; each was found to be coupled to unique intracellular signaling pathways (18). In contrast, only one PGF receptor has been cloned and sequenced; the PGF receptor contains 366 amino acids, resulting in a calculated molecular weight of 40 kDa (27). The secondary structure of the PGF receptor is consistent with that of other G protein-coupled receptors, and transfection of COS cells with cDNA for this receptor resulted in PGF₂α-stimulated activation of the PI-signaling pathway (27). The data described in the present report have confirmed that comparable PGF receptor-coupled signaling mechanisms are used during PGF₂α-stimulation of phasic myometrial contractions. Perspectives. To date, the intracellular mechanisms underlying PGF₂α-stimulated phasic myometrial contractions have not been well defined. The studies described in our report continue to address this situation. Our studies have provided evidence supporting the hypothesis that PGF₂α stimulation of the PI-signaling pathway results in the generation of cytosolic calcium oscillation-like phenomena. These intermittent calcium transients, arising from both the release of intracellular calcium and the influx of extracellular calcium, appear to result in the activation of the contractile proteins and the generation of phasic myometrial contractions. We have reported similar signal transduction phenomena occurring in response to other uterotonic agonists, including oxytocin, aluminum fluoride (a direct G protein agonist), moderate elevation of extracellular potassium (10–30 mM), ionomycin (a calcium ionophore), BAY K 8644 (a calcium channel agonist), and others (21–24). All of these observations suggest that there is a common intracellular signaling pathway occurring in uterine myocytes that results in the consistent generation of phasic contractile activity in response to uterotonic agonists. With an understanding of these intracellular mechanisms, future studies can begin to explore the hormonal and physiological mechanisms that regulate the expression and activation of this myometrial signaling pathway. Such knowledge is essential to understand and better pharmacologically treat medically important disturbances of myometrial activity, especially preterm labor and premature delivery.

This research was funded by National Institute of Child Health and Human Development research Grants HD-22063 and HD-28506. Address for reprint requests: M. Phillippe, Dept. of Obstetrics and Gynecology (MC2050), Univ. of Chicago, 5841 S. Maryland Ave., Chicago, IL 60637.

Received 5 November 1996; accepted in final form 20 June 1997.

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