Effects of Prostaglandin F₂α on the Spontaneous Contractions and Electrical Activities in the Circular Smooth Muscle of the Guinea-Pig Stomach

Sung Joon Kim, Sang Jin Lee, Byung Chul Yoon and Ki Whan Kim

Department of Physiology and Biophysics, and Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-799, Korea, and Department of Physiology, College of Medicine, Chungbuk National University

Abstract: Effects of PGF₂α and indomethacin on the mechanical and electrical membrane properties of guinea-pig antral smooth muscle were studied using microelectrodes and tension recording technique and whole cell patch clamp technique. In this tissue, indomethacin inhibited the contractility and such an effect was reversed by PGF₂α. PGF₂α increased spontaneous contractions potently and the frequency and amplitudes of slow waves were also enhanced. PGF₂α-induced contractions were not abolished by the pretreatment with nifedipine or by the nominal Ca²⁺-free condition but were abolished by pretreatment with La³⁺ or absolute Ca²⁺-free condition. Ionic currents were also measured by whole cell voltage clamp technique. PGF₂α increased the size of voltage operated Ca²⁺ inward currents, and Ca-dependent K⁺ outward currents, but had no effect on sustained outward currents. From the above results, it is assumed that PGF₂α acts as an endogenous prostaglandin in the gastric antrum of the guinea-pig. PGF₂α has promoting effects on electrical activities and such effects seem to contribute to the increase of contraction, but the greatest part of the increase of contraction is due to receptor-mediated increase of [Ca²⁺]ᵢ. Several possible mechanisms were suggested for the receptor-mediated [Ca²⁺]ᵢ increase.

Key Words: PGF₂α, Smooth muscle, Slow wave, Ionic current

INTRODUCTION

Metabolism of arachidonate results in the production of several prostaglandins (PGs) in gastric smooth muscle and mucosa. Gastric smooth muscle produces mainly PGI₂, PGE₂, PGF₂α, PGD₂. Among these products, the
biological effects of PGF$_2\alpha$ and PGE$_2$ are dominant (Bennet et al. 1977; Leduc and Needleman 1979; Lands 1979; Sanders and Northrup 1983; Coleman et al. 1990). The physiological role of PGs in regulating the electrical and mechanical behaviors of smooth muscle has been investigated by many researchers (Mishima and Kuriyama 1976; Main and Whittle 1975; Bennet et al. 1977, 1981; Sanders 1978, 1984; Sanders and Szurzewski 1981; Ishizawa 1981, 1983, 1991; Sanders et al. 1983; Kim et al. 1985; Coleman and Parkington 1985), but is not fully understood and has sometimes shown contradictory results. In canine antral circular muscle, endogenous PGs decreased the force of contractions by decreasing maximum depolarization of the slow wave (Sanders and Szurzewski 1981; Sanders 1983; Sanders et al. 1984). And also, it is said that endogenous PGs regulate the frequency of slow waves (Sanders and Szurzewski 1981; Sanders 1983; Sanders et al. 1984; Kim et al. 1985). Compared to their potent biological effects, PGs have very short half-lives. So they have been considered to be local regulatory agents in the control of gastrointestinal motility.

Apart from the actions of PGs on the mechanisms of diarrhea and the inflammatory reaction of the gastrointestinal system (Okada et al. 1989; Ueki et al. 1988; Goldman et al. 1988; Dooley et al. 1985), there are many clinical reports of motility disorders due to the long-term medication of NSAID (non-steroidal anti-inflammatory drugs) and their improvement by the application of PGs (Takeuchi and Nobuhara 1985). Also there is a case report about a patient who underwent gastrectomy to relieve conflicting gastric symptoms due to severe gastric arrhythmia, and the abnormal contractility was almost completely restored to normal by the application of indomethacin (Sanders et al. 1983).

Electrophysiological studies of the gastric musculature have been performed for many years, but compared with the progress in other excitable tissues, the field has been slow to mature. This is because the stomach, which performs unique functions in digestion, is activated by a complicated series of electrical events (Bolton 1989). Such a situation was largely improved by the development of voltage-clamp technique using patch-clamp method developed by Neher & Sakmann (Hamill et al. 1981).

Experiments were conducted to examine the regulatory role of endogenous PGs in guinea-pig's antral muscles. The effects of PGF$_2\alpha$ on isometric contraction and membrane potential were investigated and the membrane currents were also measured in single smooth muscle cells using the whole cell patch clamp method to clarify the underlying mechanism of PGF$_2\alpha$ on gastric contractility.

MATERIALS AND METHODS

Preparation of tissue and intracellular recording

Albino guinea-pigs of either sex, weighing 200-250 g, were stunned and bled. The stomach was isolated and cut in the longitudinal direction along the lesser curvature. The contents of the stomach were removed, and the mucosal layer was separated from the muscle layers in phosphate-buffered Tyrode solution (NaCl 147, KCl 4, MgCl$_2$.6H$_2$O 1.05, CaCl$_2$.2H$_2$O 2, NaH$_2$PO$_4$·2H$_2$O 0.42, Na$_2$HPO$_4$.12H$_2$O 1.81, glucose 5.5 mM, pH 7.35) at room temperature. Strips of muscle (2 mm wide, 10 mm long) were cut parallel to the circular fibers, and set in a 100ml vertical chamber. One end was fixed and the other was connected to the force transducer to measure isometric contraction (Fig.1-A).

Another strip was mounted in a 2 ml horizontal chamber. The strips were pinned out at one end with tiny pins on a rubber plate, and the other end was connected to the force transducer (Harvard)(Fig.1-B). The strip was constantly perfused at the rate of 2-3 ml/min with tris-buffered normal Tyrode solution (NaCl 147, KCl 4, CaCl$_2$.2H$_2$O 2, MgCl$_2$.6H$_2$O 1.05, tris·HCl 5, glucose 5.5 mM, pH 7.35) bubbled with 100%
dissected from the longitudinal layer and small segments of the tissues were made. The muscles were incubated in Ca^{2+}-free PSS (physiological salt solution) for 30 min at room temperature. Then, small segments were incubated for 20-30 min in Ca^{2+}-free PSS containing 0.1 % collagenase, 0.1 % trypsin inhibitor, and 0.2 % bovine serum albumin at 35°C. After digestion, single cells were dispersed by gentle agitation with a glass pipette in the Krafts-Brühe (KB) solution. Isolated gastric myocytes were kept in KB medium at 4°C. All experiments were carried out within 12 hours of harvesting cells and performed at room temperature (Fig. 2).

Membrane currents measurement: Isolated cells were transferred to a small chamber on the stage of an inverted microscope (Olympus IMT-2). The chamber was perfused with PSS (2-3 ml/min). Glass pipettes with a resistance of 2-5 MΩ were used to make a giga seal of 5-10 GΩ. Standard patch clamp techniques were used (Hamill et al. 1981). An axopatch-1C patch-clamp amplifier (Axon instrument) was used to record membrane currents. The data were displayed on a digital oscilloscope (Philips, PM3350), a pen recorder (Gould, Recorder220) and stored on videotape recorder (Victor, BR-6400) with a pulse code modulator (NF, RP-880).

Solutions: Ca^{2+}-free PSS contained (mM) NaCl 134.8, KCl 6.2, CaCl₂ 0, glucose 12.2, HEPES 0.4 and pH was adjusted to 7.3. PSS contained 2.3 mM CaCl₂ in the Ca^{2+}-free PSS. KB solution contained (in mM) L-glutamate 50, KCl 50, Taurine 20, KH₂PO₄ 20, MgCl₂ 3, glucose 10, HEPES 10, EGTA 0.5 and pH was adjusted to 7.3 by KOH. Pipette solution consisted of (in mM) K⁺-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 5, KCl 20, MgCl₂ 1, ethyleneglycol-bis (β-aminoethyl ether)-N,N,N',N'- tetraacetic acid (EGTA) 0.1, HEPES 5, pH 7.4. For studies in which K⁺ currents were blocked, pipette solution contained (in mM) Cs-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 2.5, di-Na-creatine phosphate 2.5, MgCl₂ 1, HEPES 0.1, tetraethylammonium (TEA)-Cl 20, EGTA 5, pH 7.4.
RESULTS

1. Effects of PGF$_2$α on the contractility of antral circular muscle strips

PGF$_2$α increased both the amplitude of spontaneous contractions and the basal tone dose-dependently from the low concentration (5pM, Fig. 3). The maximal contraction was observed at $5 \times 10^{-7}$M. Above the maximal level, PGF$_2$α showed rather inhibitory effects on the contractility. Usually, the frequency of spontaneous contractions was accelerated by PGF$_2$α (from 4.2/min to 6.6/min at 1 μM in this case).

As the pretreatment with TTX (tetrodotoxin, 0.1 μM) did not change the effects of PGF$_2$α, we could exclude the possibility of any indirect action of PGF$_2$α via the intrinsic nervous system (Fig. 4).

2. Effects of PGF$_2$α on the membrane potential and slow waves of antral circular muscle strips

PGF$_2$α depolarized the membrane potential slightly and increased the amplitude of slow waves (25%, 10%, 13%, 30% in 4 experiments) and depolarizing velocity (Fig. 5-A). Both the abortive spikes and plateau phase were raised by PGF$_2$α and such effects were accompanied by the enhancement of contractility as previously shown (Fig. 5-B).

![Fig. 2. Light microscopic appearance of a single gastric myocyte obtained from the guinea-pig stomach. A microelectrode tip has been approached to make a giga-seal.](image-url)
Fig. 4. PGF₂α did not act indirectly through the enteric nerve system. The excitatory effect of the PGF₂α (0.5 μM) on the spontaneous contractions (A) was not influenced by pretreatment with TTX (tetrodotoxin, 0.1 μM), ATR (atropine, 1 μM) and GND (guanethidine, 2 μM) (B).

3. Effects of indomethacin on the membrane potential and spontaneous contractions

To observe the influence of the endogenous PGs on the contractility, we applied indomethacin (10 μM). Indomethacin usually had an inhibitory effect on spontaneous contractions (Fig.6) and slow waves (decreased the frequency and size of abortive spikes at the peak of slow waves, Fig.7-A,B). Such effect was restored by the application of PGF₂α. But sometimes, indomethacin had little effect on slow waves (Fig.7-C).

4. Dependency of the effect of PGF₂α on the Ca²⁺ influx

When we applied nifedipine which is known to block the L-type voltage-operated Ca²⁺ channel, spontaneous contractions were extinguished. But in this condition, PGF₂α still could induce a substantial degree of contraction (Fig.8-A). In another tissue, we applied La³⁺ (30 μM), known as one of the most potent inorganic
Ca²⁺ channel blockers (Evans 1988). At this condition, PGF₂α could not change the contractility (Fig.9-A). Acetylcholine, which potentiates the contractility mainly through the IP₃-mediated Ca²⁺-release (Rasmussen et al. 1990), also showed a similar tendency (Fig.8-B, 9-B).

PGF₂α could still induce contraction when there remained little spontaneous contraction at the nominal Ca²⁺-free condition (simple omission of external Ca²⁺, Fig.10-B). But as we eliminated the external Ca²⁺ more and more completely using EGTA (Ethylenglycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid, relatively specific Ca²⁺ chelator), we could not
of the single smooth muscle cells of the antrum. Electrophysiological properties of the isolated myocytes were studied by the usual whole cell patch clamp technique (Hamill et al. 1981). For recording only inward currents, we used cesium-aspartate solution with 20mM TEA-Cl in the pipette. (Mitchell et al. 1987). Under this condition, mainly inward currents were observed by clamping membrane potentials from holding potential (−60mV) to various levels (−40−50mV) of stimulating potentials (Fig. 11). Because TTX (tetrodotoxin) had no effect on the shape of these inward currents (data not shown here), we did not use TTX to exclude Na+-currents. The inward currents were proved to be composed of only L-type voltage operated Ca2+-current (L-VOCC) because, 1) the currents were

see the effect of PGF2α (Fig. 10-C,D).

5. Effects of PGF2α on the whole cell ionic currents

1) Voltage operated Ca2+ inward currents (VOCCs, I_{Ca})

Isolated single smooth muscle cells were 5-10 μm in diameter and 100-200 μm in length. Fig. 2 shows the light microscopic appearance of the single smooth muscle cells of the antrum. Electrophysiological properties of the isolated myocytes were studied by the usual whole cell patch clamp technique (Hamill et al. 1981). For recording only inward currents, we used cesium-aspartate solution with 20mM TEA-Cl in the pipette. (Mitchell et al. 1987). Under this condition, mainly inward currents were observed by clamping membrane potentials from holding potential (−60mV) to various levels (−40−50mV) of stimulating potentials (Fig. 11). Because TTX (tetrodotoxin) had no effect on the shape of these inward currents (data not shown here), we did not use TTX to exclude Na+-currents. The inward currents were proved to be composed of only L-type voltage operated Ca2+-current (L-VOCC) because, 1) the currents were

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Fig. 12. Effects of verapamil or Ca\textsuperscript{2+}-removal on Ca\textsuperscript{2+} currents. Treatment with verapamil (5 \textmu M) left little inward current (left), and the same result was obtained at Ca\textsuperscript{2+}-free condition.

completely blocked by the removal of Ca\textsuperscript{2+} in the bath solution (Fig.12) or 2) by the application of nifedipine or verapamil (Fig.12) and 3) changing holding potential to more negative (-80mV) did not reveal any T-type like Ca\textsuperscript{2+} current which is activated at a more negative (lower than -40mV) voltage area (Fig 13).

Voltage-operated calcium inward currents (VOCCs, \textit{i}_{Ca}) and PGF\textsubscript{2\alpha}

A family of \textit{i}_{Ca} activated by different depolarizing pulses from a holding potential of -65mV is shown in Fig. 14. \textit{i}_{Ca} became apparent near -30mV, reached its maximum at +10mV, and decreased at more positive membrane potentials. In the same cell, PGF\textsubscript{2\alpha} increased the inward currents (Fig.14). Typical current-voltage relationships for \textit{i}_{Ca} of this experiment are also shown. Although the increase of inward currents were not so prominent, such increase was consistent in other cells (18.5%, 20%, 40%, 23% in 4 cases).

2) Potassium outward currents
To record Ca-dependent K\textsuperscript{+}-outward current (\textit{i}_{KCa}), intrapipette solution was changed to high K\textsuperscript{+}-low EGTA (0.1mM)-solution. Depolarization of cells between -30~10mV from the holding potential of -60mV during 400 miliseconds elicited a large initial transient and following oscillatory outward currents superimposed on sustained outward current (Fig. 15). These spontaneous transient outward currents (STOCs, Benham & Bolton,1986) have been described in many smooth muscle cells studied and at present these currents are considered to be elicited by the activation of Ca-dependent K\textsuperscript{+} channels by Ca\textsuperscript{2+} released from the sarcoplasmic reticulum (Benham & Bolton, 1986; Ohya et al., 1987; Bolton & Lim, 1989). To characterize these transient outward currents further, we increased the concentration of EGTA in the pipette solution (designed to strongly buffer intracellular Ca\textsuperscript{2+}), the STOCs were abolished almost completely (Fig.16). The remaining outward currents are mainly so-called delayed rectifier outward currents, because they are relatively slow to reach their peak value.

3) Potassium outward currents and PGF\textsubscript{2\alpha}
Fig. 17 shows the effects of PGF\textsubscript{2\alpha} on the \textit{i}_{KCa} produced by commanding pulses from
Fig. 13. Effect of varying holding potential on Ca\textsuperscript{2+} currents. (A) Currents were elicited from holding potential of \(-50\text{mV}\) (upper traces) to test potentials between \(-40\) and \(50\text{mV}\) with \(10\text{mV}\) increment and from holding potential of \(-80\text{mV}\) (lower traces). (B) Current-voltage relationships for the peak currents recorded from \(-50\text{mV}\) (filled circles) and \(-80\text{mV}\) (hollow circles) respectively. There was no difference between the threshold potential for the activation of inward current in spite of the increase of inward currents.

\(-60\text{mV}\) to various levels (\(-30\)–\(-30\text{mV}\)) with the duration of 400 ms. By the application of PGF\textsubscript{2α} (0.3 μM) to the bath solution, transient and spontaneous oscillatory outward currents were increased, suggesting the increase of intracellular [Ca\textsuperscript{2+}]. But PGF\textsubscript{2α} had no effect on the delayed rectifier outward currents which are the remaining outward currents when intracellular Ca\textsuperscript{2+} was clamped almost to zero with high EGTA (Fig.18).

**DISCUSSION**

The contractions of the gastrointestinal smooth muscles are initiated from smooth muscle cells themselves. In other words, the regulation of gastrointestinal motility depends upon the myogenic activity, namely slow waves. And slow waves are modulated by intrinsic and extrinsic nerves as well as by circulating hormones and drugs (Suzuki and Kuriyama 1975).
Although the mechanism of smooth muscle contraction is quite different from that of skeletal muscle contraction, the increase of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) is still necessary. The increase of Ca\(^{2+}\)-activated K\(^+\) current seems to reflect the enhancement of [Ca\(^{2+}\)]\(_i\) increase (Fig. 17). Two integrated membrane systems are involved in the control of smooth muscle [Ca\(^{2+}\)]\(_i\): (a) the plasmalemma, which is under the control of the membrane potential (Em) and agonists such as neurotransmitter hormones and autacoids, and (b) the sarcoplasmic reticulum, which is under the control of second messengers (van Breemen and Saida 1989).

The data presented here suggest that PGF\(_{2\alpha}\) is the main endogenous PGs from guinea-pig gastric antral myocyte (Fig. 6, Fig. 7) and has a 2-fold regulatory role in this tissue: 1) excitatory influence on the strength of contraction and slow waves 2) accelerating influence on the frequency of spontaneous contractions.

PGs, like other local hormones, produce their effects by interacting with specific receptors on cell membrane. Even in the absence of direct evidence, the presence of such receptors may be inferred from the properties of prostanoids. For example, (1) PGs usually have high potency, (2) small chemical modifications can have profound effects on their potency and profile of biological activity, (3) different prostanoids can have different effects on the same cell type. Now, it is possible to say that distinct receptors exist for each of the five naturally occurring prostanoids, PGD, PGE\(_2\), PGF\(_{2\alpha}\), PGI\(_2\), TxA\(_2\) (DP, EP, FP, IP, TP; respectively). But they are not so exclusively specific between each group (Coleman et al. 1990). Furthermore, evidence is beginning to accumulate that, at least in the case of receptors for PGE\(_2\) and TxA\(_2\), further subdivision is possible. Among these, at least four prostanoid receptor types can mediate the contraction of smooth muscle, i.e. EP\(_1\), EP\(_3\), TP and FP. Direct evidence that the activation of FP receptors mobilizes Ca\(^{2+}\) is difficult to find and contraction of vascular smooth muscle by PGF\(_{2\alpha}\) is believed to be mediated by TP receptors. Activation of TP receptors causes contraction of smooth muscle by increasing intracellular free Ca\(^{2+}\) which is
achieved by both opening receptor-operated channels in the plasma membrane and mobilizing intracellular Ca\(^{2+}\) from the sarcoplasmic reticulum. Although the exact mechanism of the latter effect is not entirely clear, it could be triggered by influx of extracellular Ca\(^{2+}\) and/or involve phosphatidyl inositol turnover (Coleman et al. 1990; Loutzenhiser et al. 1981; Toda 1982; Bertaccini and Coruzzi 1987).

In guinea-pig's antrum, PGF\(_2\alpha\) increased the size of slow waves, depolarizing velocity and number of abortive spikes (Fig. 5). Some features of these results can be attributed to the increase of voltagel operated Ca\(^{2+}\) current (VOCC) by PGF\(_2\alpha\). So, part of the PGF\(_2\alpha\)-induced contraction is due to the enhancing effect on VOCC and slow waves. But as we have seen, nifedipine, one of the inhibitors for L-type VOCC, could not completely block the effect of PGF\(_2\alpha\) on contractility (Fig. 8), so there must be some other pathways to increase the \([\text{Ca}^{2+}]_i\). As in other reports (Katzka and Morad 1989; Noack et al. 1992), we have not found any T-type VOCC from the muscle cells of guinea-pig antrum (Fig. 12, Fig. 13), so there remains two possibilities; 1) activation of Ca\(^{2+}\)-influx pathway other than VOCC, 2) activation of second messenger-mediated (IP\(_3\) or Ca\(^{2+}\) mediated) stored Ca\(^{2+}\)release. It is suggested that activation of TP receptors can induce both effects (Coleman et al. 1990).

As the pretreatment with La\(^{3+}\) or the complete elimination of Ca\(^{2+}\) from bath solution could inhibit the PGF\(_2\alpha\)-induced contraction (Fig. 9, Fig. 10), the first possibility is preferable. Depending on the cell type and on the agonist, several hypotheses have been advanced but the mechanisms and regulation of receptor operated Ca\(^{2+}\) influx are still controversial and the nature of the calcium influx pathway is still unknown. One hypothesis, called ‘capacitive Ca\(^{2+}\) entry’ argues that depletion of intracellular Ca\(^{2+}\) stores provides a signal to allow Ca\(^{2+}\) entry which is used to replenish the intracellular Ca\(^{2+}\) stores (Jacob 1990). Another possibility is that certain hormone receptors might directly open channels admitting Ca\(^{2+}\) but direct evidence for this has been found only in a few cases in smooth muscle; ATP receptors in vascular smooth muscle (Benham & Tsien 1987) and muscarinic receptors in G-I smooth muscle (Inoue 1991). Last, it is possible that receptors might activate the Ca\(^{2+}\)-transport system through the mediation of second messengers, such as Ca\(^{2+}\) (Von Tscharner et al. 1986), InsP\(_3\) (Kuno and Gardner 1987) or the combination of InsP\(_3\) with its phosphorylated product inositol 1,3,4,5-
Fig. 17. The effect of PGF\(_2\alpha\) on the calcium-dependent potassium outward currents. Transient and oscillatory outward currents were elicited by the depolarizing pulses to -30, -15, 0, 15, 30 mV from the holding potential of -60 mV, using a high K\(^+\) solution containing 0.1 mM of EGTA in the pipette. These outward currents were enhanced by PGF\(_2\alpha\) (0.3 \(\mu\)M) both in amplitude and frequency.

Fig. 18. The effect of PGF\(_2\alpha\) on the voltage dependent delayed outward potassium current. The voltage-dependent sustained outward currents were elicited by the depolarizing pulses to -30, -15, 0, 15, 30 mV from the holding potential of -65 mV using a high K\(^+\) solution containing 10 mM of EGTA in the pipette. These currents were not changed by the application of PGF\(_2\alpha\) (0.5 \(\mu\)M).

tetakisphosphate (Irvine and Moor 1986). But the above hypotheses are still controversial and beyond the aim of this experiment, so we did not go any further into these possibility.

Thinking about the second possibility, we see that two different calcium release mechanisms have been detected in the calcium store in smooth muscle cells by using skinned fiber preparations (Endo et al. 1990). One is a mechanism activated by applying calcium ion itself (CICR), and the other is that activated by inositol-trisphosphate (IP\(_3\), IICR). IICR could be evoked in the practical absence of Ca\(^{2+}\), but CICR was activated by Ca\(^{2+}\) only in
concentrations above micromolar range (10 times higher than the resting level of $[Ca^{2+}])$. So, those results which showed extracellular $Ca^{2+}$-dependency of PGF$_2\alpha$-induced contraction (Fig. 10) also suggest the first possibility, that PGF$_2\alpha$ enhanced CICR. But as it is now widely accepted that inositol 1,4,5-trisphosphate (InsP$_3$) is the intracellular messenger which links cell surface receptor stimulation to the release of intracellular $Ca^{2+}$ pools (Berridge 1987; Hashimoto et al. 1987), the possibility of IIICR should be considered cautiously, too. Recently it has been reported that heavy metal ions, like Cd$^{2+}$, inhibited not only $Ca^{2+}$ via VOCC,ROCC and Na/Ca exchange, but also intracellular $Ca^{2+}$ release from the sarcoplasmic reticulum in the antral circular muscle of guinea-pig stomach (Kim and Han 1992). And in our results, La$^{3+}$ inhibited ACh-induced as well as PGF$_2\alpha$-induced contraction (Fig.9). ACh is the representative neurotransmitter which acts through the IP$_3$-mediated $Ca^{2+}$-release from sarcoplasmic reticulum (Rasmussen et al. 1990). So there is a possibility that pretreatment with La$^{3+}$ might have inhibited the IIICR induced contraction by PGF$_2\alpha$ and such a possibility should be considered cautiously. Maybe, $[Ca^{2+}]_i$ measurement by fluorescent dye would be helpful in clarifying the source of the nifedipine insensitive pathway of calcium increase by PGF$_2\alpha$.

From the above results it is suggested that PGF$_2\alpha$ is an endogenously released and physiologically acting prostaglandin. The excitatory action of PGF$_2\alpha$ on contractility is partly due to the increase of nifedipine sensitive VOCC, but the nifedipine insensitive pathways of PGF$_2\alpha$ action have not been clarified yet. Data suggest both the receptor operated $Ca^{2+}$-influx and the enhancement of $Ca^{2+}$-release.

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