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Effects of Prostaglandin $F_2\alpha$ on the Spontaneous Contractions and Electrical Activities in the Circular Smooth Muscle of the Guinea-Pig Stomach†

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=Abstract=**Effects of $PGF_2\alpha$ and indomethacin on the mechanical and electrical membrane properties of guinea-pig antral smooth muscle were studied using microeletrodes and tension recording technique and whole cell patch clamp technique. In this tissue, indomethacin inhibited the contractility and such an effect was reversed by $PGF_2\alpha$. $PGF_2\alpha$ increased spontaneous contractions potently and the frequency and amplitudes of slow waves were also enhanced. $PGF_2\alpha$-induced contractions were not abolished by the pretreatment with nifedipine or by the nominal Ca^{2+}-free condition but were abolished by pretreatment with La^{3+} or absolute Ca^{2+}-free condition. Ionic currents were also measured by whole cell voltage clamp technique. $PGF_2\alpha$ increased the size of voltage operated Ca^{2+} inward currents, and Ca-dependent K^+ outward currents, but had no effect on sustained outward currents. From the above results, it is assumed that $PGF_2\alpha$ acts as an endogenous prostaglandin in the gastric antrum of the guinea-pig. $PGF_2\alpha$ 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 $[Ca^{2+}]_i$. Several possible mechanisms were suggested for the receptor-mediated $[Ca^{2+}]_i$ increase.**

Key Words: *$PGF_2\alpha$, 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 PGI_2,PGE_2, $PGF_2\alpha$,PGD_2. Among these products, the

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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 Szurszewski 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; Dooly *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 intracelluar 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, $MgCl_2.6H_2O$ 1.05, $CaCl_2.2H_2O$ 2, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4.12H_2O$ 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, $CaCl_2 \cdot 2H_2O$ 2, $MgCl_2 \cdot 6H_2O$ 1.05, tris · HCl 5, glucose 5.5 mM, pH 7.35) bubbled with 100 %

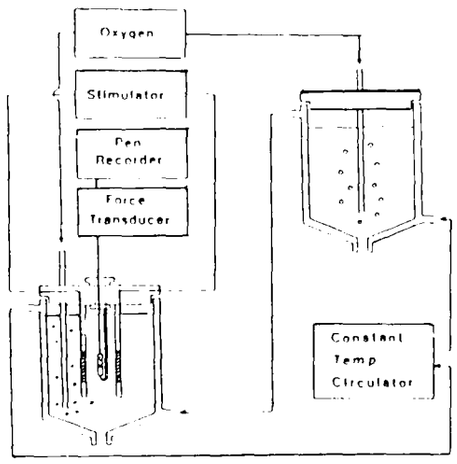


Fig. 1. (A) A schematic representation of the 100 ml vertical chamber and the isometric recording system. The chamber was maintained at a constant temperature and saturated with 100% O_2.(B) A schematic representation of the simultaneous recording system for isometric contractions and electrical activities. The intracellular microelectrode was used to record the changes of membrane potential, while simultaneously recording isometric contractions by the tension transducer. *CRO: cathode ray oscilloscope

O_2 and maintained at 35°C. Electrical responses of smooth muscle cells were recorded by means of glass microelectrodes filled with 3 M KCl. Microelectrodes with a tip resistance of 40-80 MΩ were used only. Mechanical and electrical responses of smooth muscle cells were simultaneously recorded on a Device pen recorder.

Patch clamp experiment

Isolation of cells: The circular muscle was 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, $CaCl_2$ 0, glucose 12.2, HEPES 0.4 and pH was adjusted to 7.3 by tris. PSS contained 2.3 mM $CaCl_2$ in the Ca^{2+}-free PSS. KB solution contained (in mM) L-glutamate 50, KCl 50, Taurine 20, KH_2PO_4 20, $MgCl_2$ 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, $MgCl_2$ 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, $MgCl_2$ 1, HEPES 0.1, tetraethylammonium (TEA)-Cl 20, EGTA 5, pH 7.4.

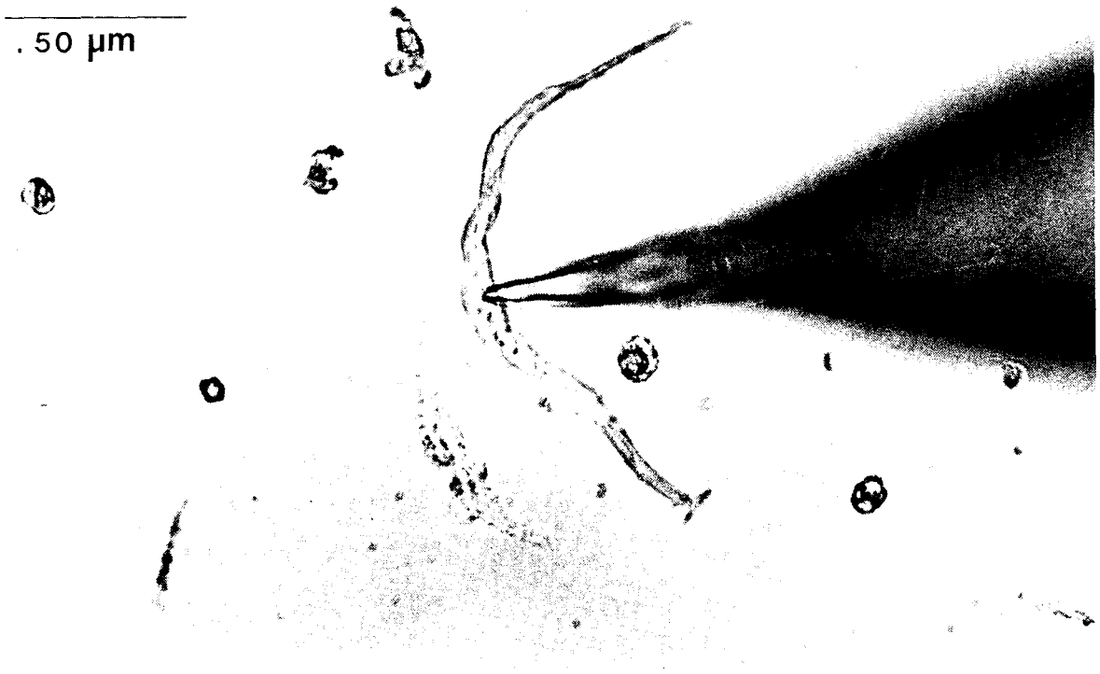


Fig. 2. Light microscopic appearance of a single gastric myocyte obtained from the guinea-pig stomach. A microelectrode tip has been approached to make a giga-seal.

RESULTS

1. Effects of $PGF_2\alpha$ on the contractility of antral circular muscle strips

$PGF_2\alpha$ 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 5×10^{-7}M. Above the maximal level, $PGF_2\alpha$ showed rather inhibitory effects on the contractility. Usually,the frequency of spontaneous contractions was accelerated by $PGF_2\alpha$ (from 4.2/min to 6.6/min at 1 μM in this case).

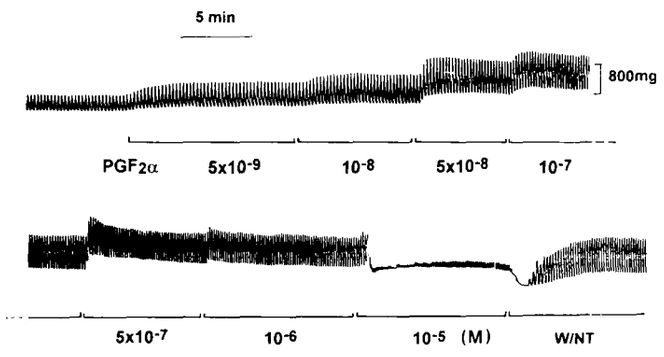


Fig. 3. Effects of $PGF_2\alpha$ on the spontaneous contractions of the antral circular muscle in guinea-pig stomach. $PGF_2\alpha$ potently enhanced both the spontaneous contractions and the basal tone dose-dependently. Maximal potentiating effects of $PGF_2\alpha$ appeared around 10^{-7}M. At the higher concentration, rather inhibitory effects were also shown.

As the pretreatment with TTX (tetrodotoxin,0.1 μM) did not change the effects of $PGF_2\alpha$, we could exclude the possibility of any indirect action of $PGF_2\alpha$ via the intrinsic nervous system (Fig.4).

2. Effects of $PGF_2\alpha$ on the membrane potential and slow waves of antral circular muscle strips

$PGF_2\alpha$ 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 $PGF_2\alpha$ and such effects were accompanied by the enhancement of contractility as previously shown (Fig.5-B).

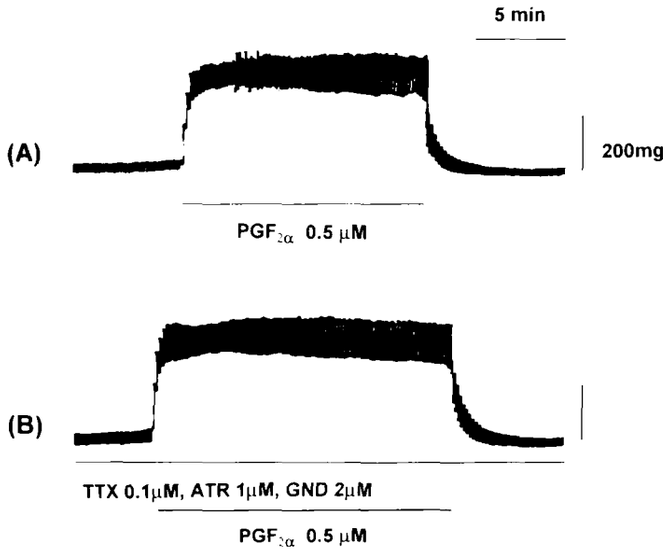


Fig. 4. $PGF_2\alpha$ did not act indirectly through the enteric nerve system. The excitatory effect of the $PGF_2\alpha$ (0.5 μM) on the spontaneous contractions (A) was not influenced by pretreatment with TTX (tetrodotoxin,0.1 μM),ATR (atropine,1 μM) and GND (guanethidine,2 μM) (B).

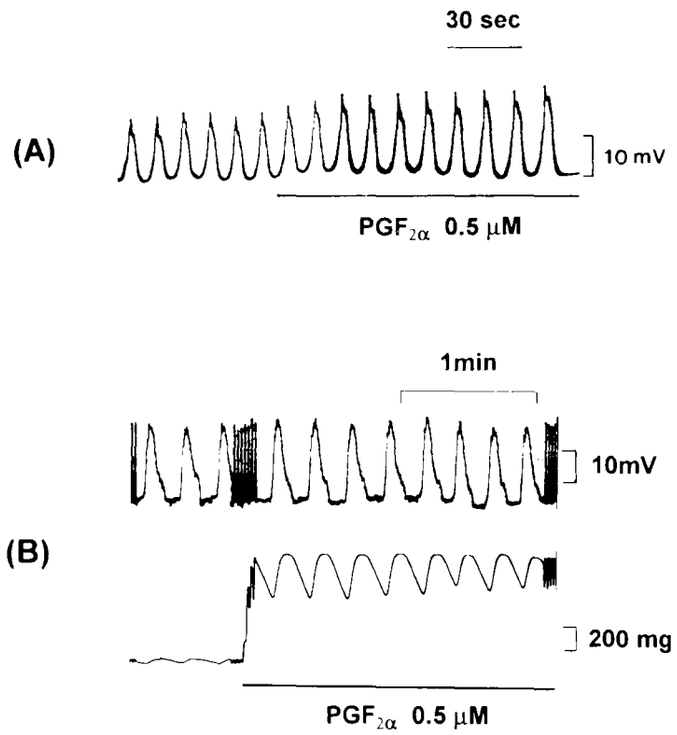


Fig. 5. Effects of $PGF_2\alpha$ (0.5 μM) on the membrane potential and slow waves of antral circular muscle strip. (A) The changes in slow wave parameters were observed: slight depolarization and the increase in the amplitude. Abortive spikes were also enhanced after the addition of $PGF_2\alpha$. (B) Plateau phases of slow waves were elevated with accompanying enhancement of contractions.

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 applicaiton of $PGF_2\alpha$. But sometimes, indomethacin had little effect on slow waves (Fig.7-C).

4. Dependency of the effect of $PGF_2\alpha$ on the Ca^{2+} influx

When we applied nifedipine which is known to block the L-type voltage-operated Ca^{2+} channel, spontaneous contractions were extinguished. But in this condition, $PGF_2\alpha$ still could induce a substantial degree of contraction (Fig.8-A). In another tissue, we applied La^{3+} (30 μM), known as one of the most potent inorganic

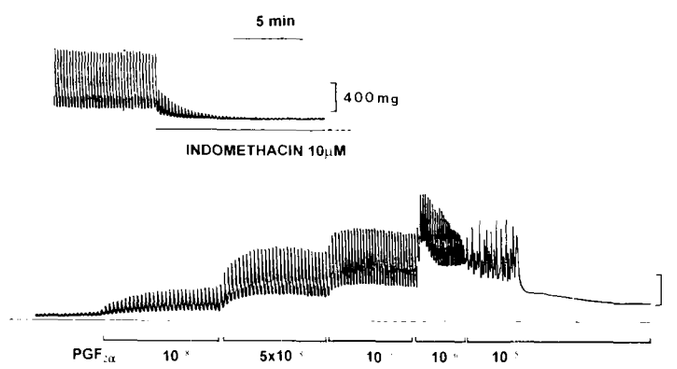
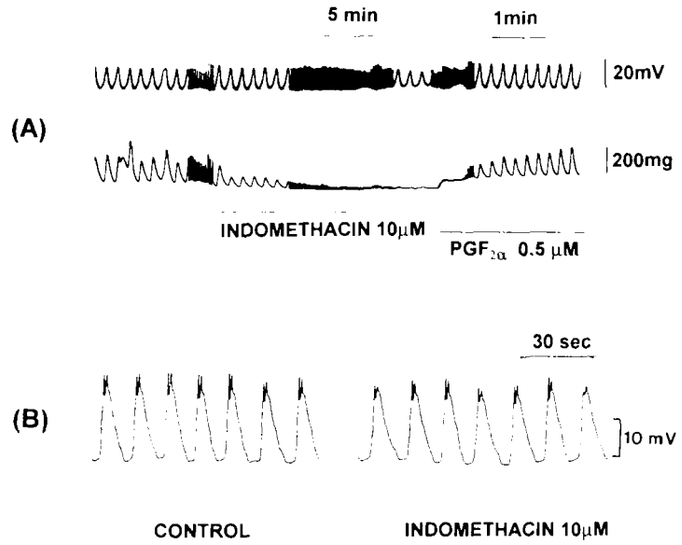


Fig. 6. Inhibitory effect of indomethacin (10 μM) on the spontaneous contractions of antral circular muscle in guinea-pig stomach. Both the amplitude of spontaneous contractions and basal tone were inhibited by indomethacin and also the frequency of spontaneous contractions was decreased. Such inhibitory effects were reversed by the addition of $PGF_2\alpha$.



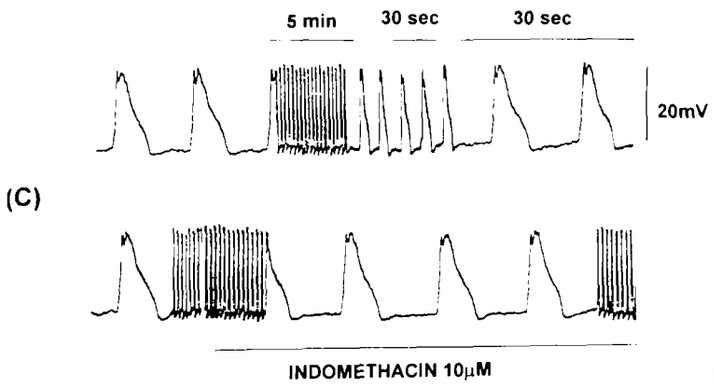


Fig. 7. Effects of indomethacin on the slow waves recorded in the antral circular muscle of guinea-pig stomach. (A) Indomethacin decreased the frequency of slow waves and the amplitudes of abortive spikes. (B) Similar results were obtained when recording both slow waves and spontaneous contractions and such effects were reversed by the application of $PGF_2\alpha$. (C) In some cases $PGF_2\alpha$ had little effect on slow waves.

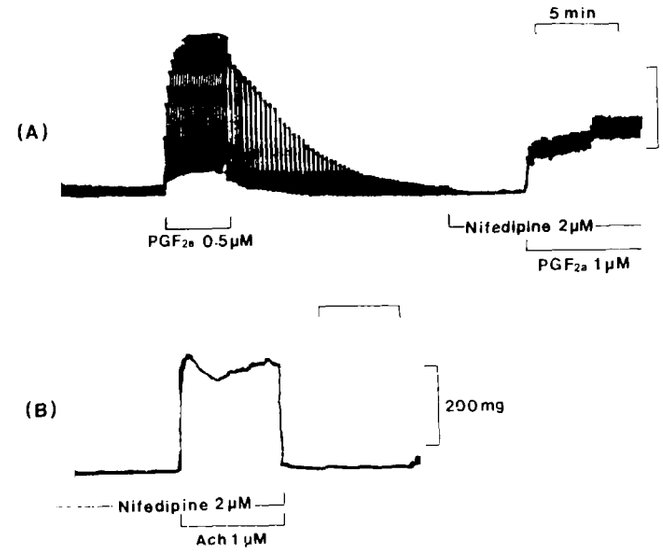


Fig. 8. Effects of nifedipine (5 μM) on the excitatory actions of $PGF_2\alpha$ and acetylcholine. The excitatory effect of $PGF_2\alpha$ on tonic and phasic contractions was not blocked by pretreatment with nifedipine. Acetylcholine also increased the tone of smooth muscle after pretreatment with nifedipine.

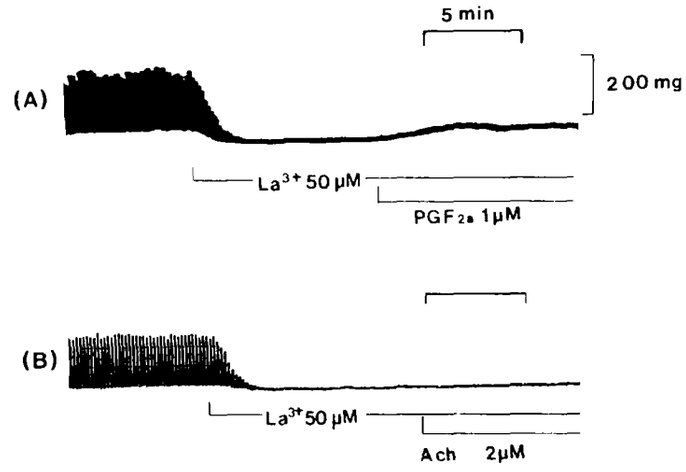


Fig. 9. Effects of lanthanum (50 μM) on the excitatory actions of $PGF_2\alpha$ and acetylcholine. After pretreatment with lanthanum, the excitatory effect of $PGF_2\alpha$ (1 μM) and acetylcholine (2 μM) was blocked almost completely.

Ca^{2+} channel blockers (Evans 1988). At this condition, $PGF_2\alpha$ could not change the contractility (Fig.9-A). Acetylcholine, which potentiates the contractility mainly through the IP_3-mediated Ca^{2+}-release (Rasmussen *et al.* 1990), also showed a similar tendency (Fig.8-B, 9-B).

$PGF_2\alpha$ could still induce contraction when there remained little spontaneous contraction at the nominal Ca^{2+}-free condition (simple omission of external Ca^{2+}, Fig.10-B). But as we eliminated the external Ca^{2+} more and more completely using EGTA (Ethyleneglycol-bis (β-aminoethylether) N,N,N′,N′-tetraacetic acid, relatively specific Ca^{2+} chelator), we could not

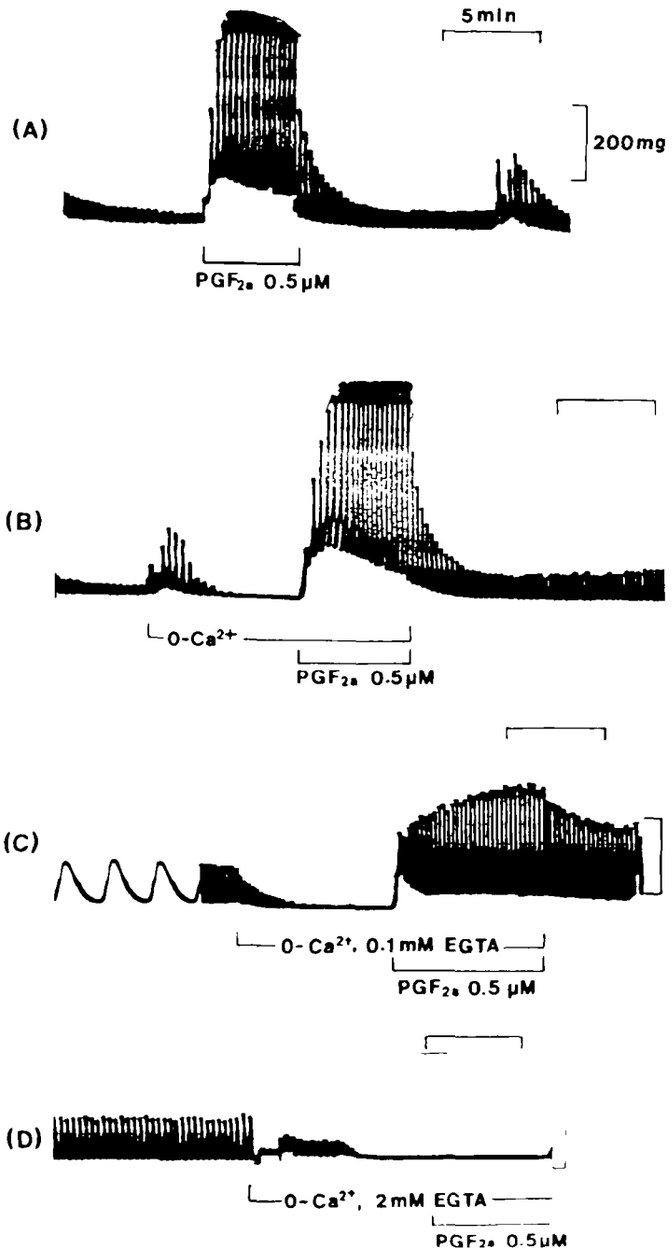


Fig. 10. Effects of external Ca^{2+} on the excitatory actions of $PGF_2\alpha$. The effects of $PGF_2\alpha$ were not inhibited at the nominal Ca^{2+}-free condition. But as the concentration of EGTA increased, excitatory effects were abolished.

see the effect of $PGF_2\alpha$ (Fig.10-C,D).

5. Effects of $PGF_2\alpha$ on the whole cell ionic currents

1) Voltage operated Ca^{2+} 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 used TTX to exclude Na^+-currents. The inward currents were proved to be composed of only L-type voltage operated Ca^{2+}-current (L-VOCC) because, 1) the currents were

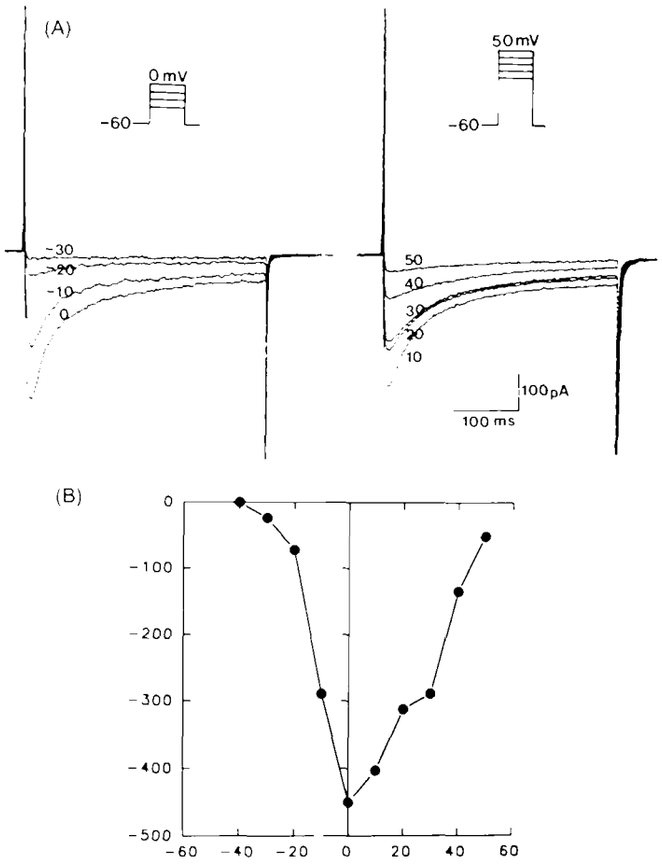


Fig. 11. A family of Ca^{2+} currents recorded at different test potentials indicated in mV near each current trace (A). The current-voltage relationship for Ca^{2+} currents measured at the peak of each current. Holding potential was -60mV.

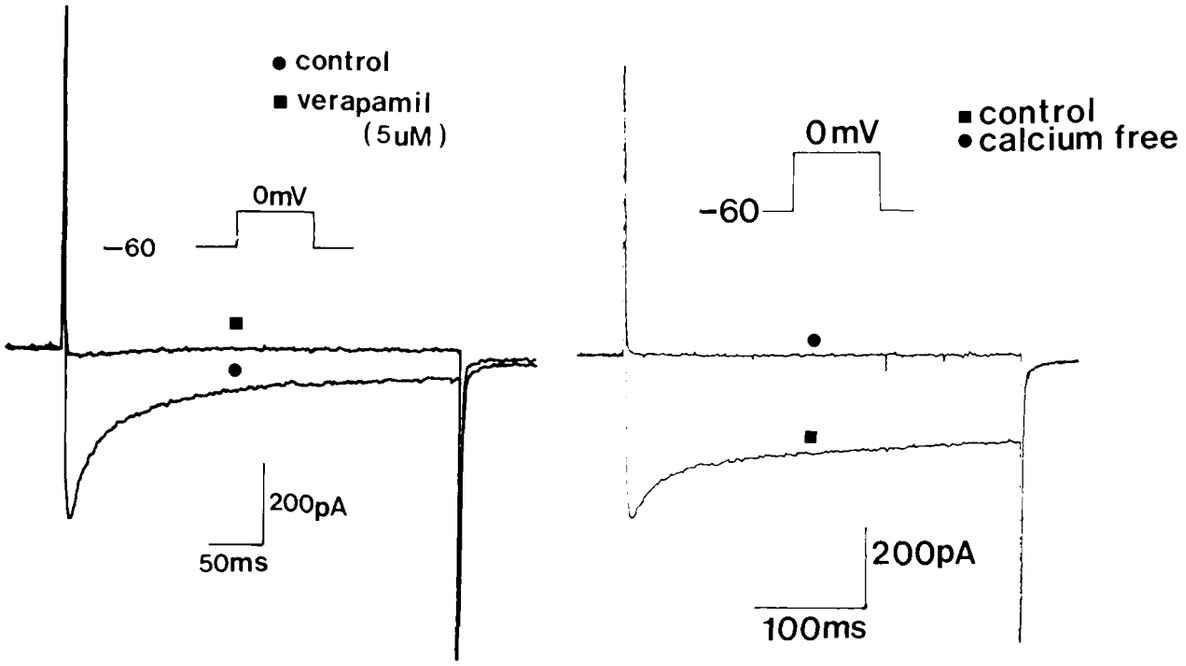


Fig. 12. Effects of verapamil or Ca^{2+}-removal on Ca^{2+} currents. Treatment with verapamil (5 μM) left little inward current (left), and the same result was obtained at Ca^{2+}-free condition.

completely blocked by the removal of Ca^{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^{2+} current which is activated at a more negative (lower than -40mV) voltage area (Fig 13).

Voltage-operated calcium inward currents (VOCCs, i_{Ca}) and $PGF_2\alpha$

A family of i_{Ca} activated by different depolarizing pulses from a holding potential of -65mV is shown in Fig. 14. I_{Ca} became apparent near -30mV, reached its maximum at +10mV, and decreased at more positive membrane potentials. In the same cell, $PGF_2\alpha$ increased the inward currents (Fig.14). Typical current-voltage relationships for I_{Ca} of this experimant are also shown. Although the increase of inward cuurents 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^+-outward current (i_{KCa}), intrapipette solution was changed to high K^+-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^+ channels by Ca^{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^{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 $PGF_2\alpha$

Fig. 17 shows the effects of $PGF_2\alpha$ on the i_{KCa} produced by commanding pulses from

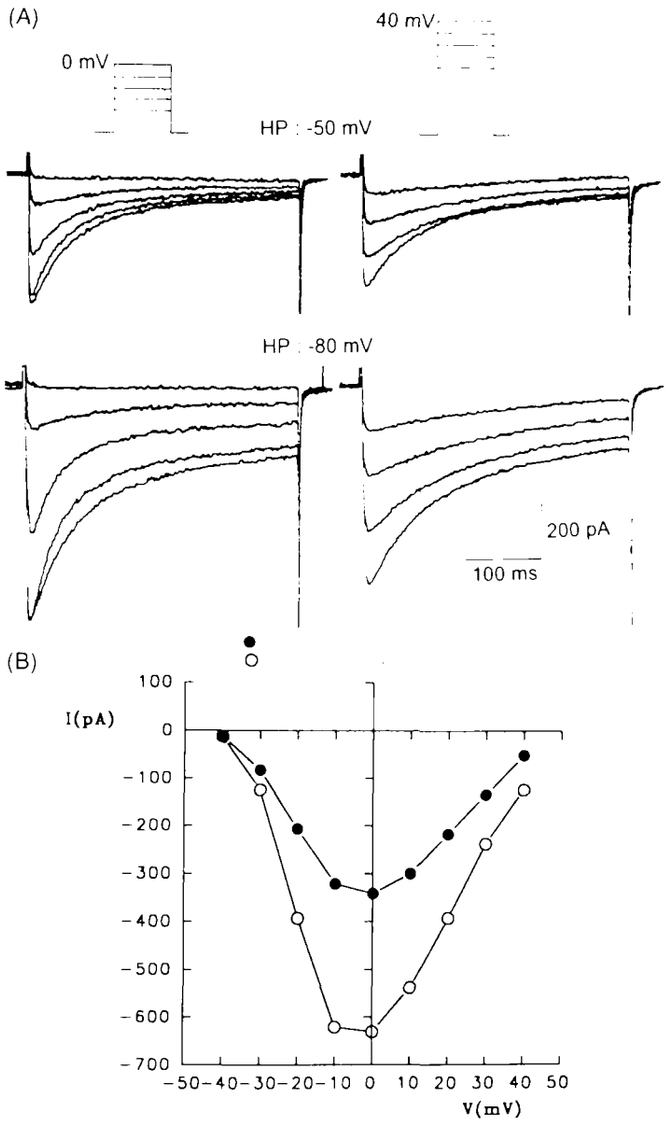


Fig. 13. Effect of varying holding potential on Ca^{2+} currents. (A) Currents were elicited from holding potential of -50mV (upper traces) to test potentials between -40 and 50mV with 10mV increment and from holding potential of -80mV (lower traces). (B) Current-voltage relationships for the peak currents recorded from -50mV (filled circles) and -80mV (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.

-60mV to various levels (-30~30mV) with the duration of 400 ms. By the application of $PGF_2\alpha$ (0.3 μM) to the bath solution, transient and spontaneous oscillatory outward currents were increased, suggesting the increase of intracellular [Ca^{2+}]. But $PGF_2\alpha$ had no effect on

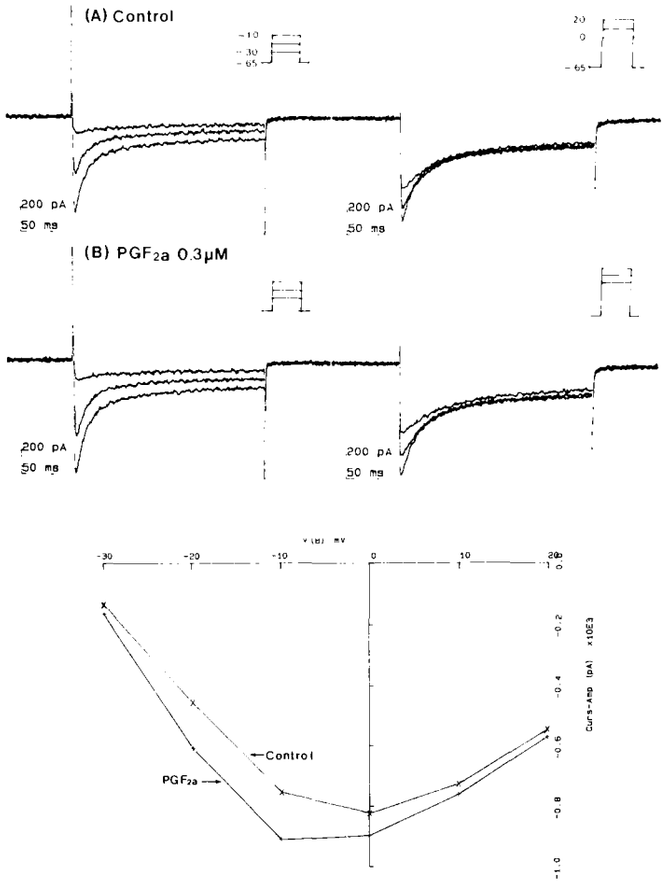


Fig. 14. The effect of $PGF_2\alpha$ (3×10^{-7}M) on Ca^{2+} currents. Ca^{2+} currents were elicited from holding potential of --5mV to test potentials between --0 and 20mV with 15mV increment (A). These currents were increased a little by the treatment with $PGF_2\alpha$ (B). Their current-voltage relationships are shown under (A) and (B).

the delayed rectifier outward currents which are the remaining outward currents when intracellular Ca^{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).

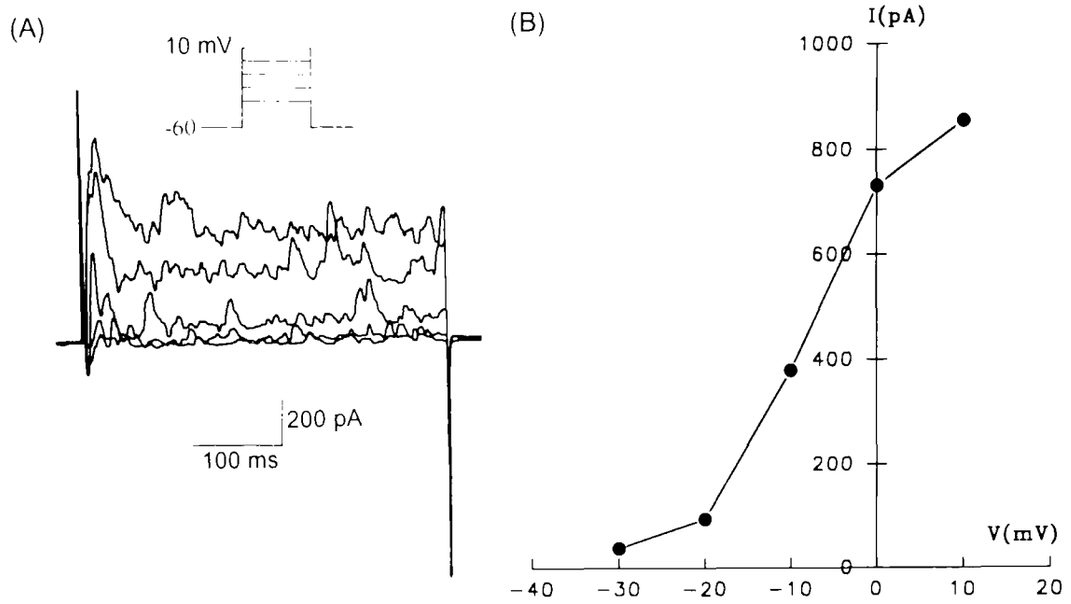


Fig. 15. Ca^{2+}-dependent potassium outward currents($i_{K(Ca)}$) elicited from holding potential of -60mV to test potentials between -30 and 10mV with 10mV increment, using high K^+ solution containing 0.1mM of EGTA in the pipette. Initial and spontaneous transient outward currents are shown superimposed upon sustained outward currents (A). Current-voltage relationship of the initial peak values are plotted (B).

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

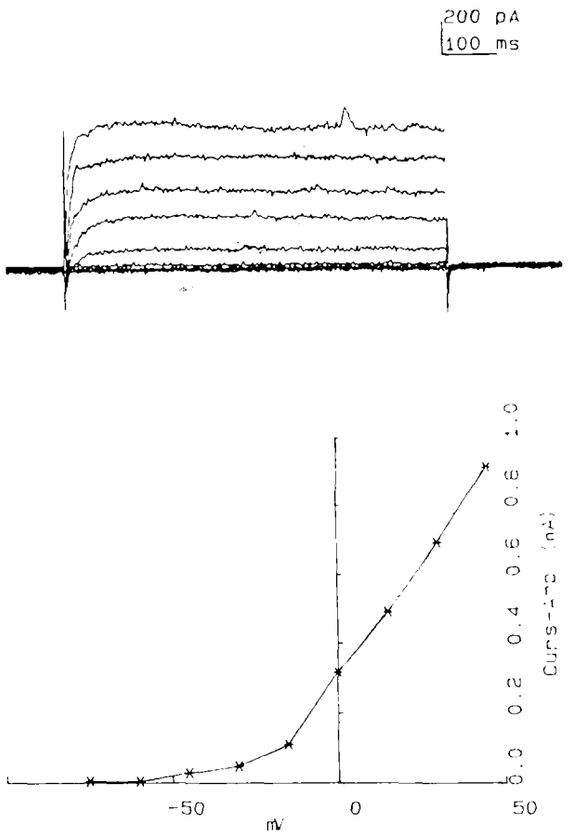


Fig. 16. Sustained outward currents recorded with high EGTA in pipette solution. (A) Outward currents were elicited from holding potential of -60mV to test potentials between -75 and 45mV with 15mV increment, using high K^+ solution containing 10 mM of EGTA in the pipette. Under these conditions, the outward currents reached the peak value after some delay (50-100ms), and inactivated very slowly. (B) Current-volatage relationship of these currents. The outward currents were activated around -50mV and showed outward rectification.

achieved by both opening receptor-operated channels in the plasma membrane and mobilizing intracellular Ca^{2+} from the sarcoplasmic reticulum. Although the exact machanism 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 volatage 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 $[Ca^{2+}]_i$. 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 mechnisms 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-

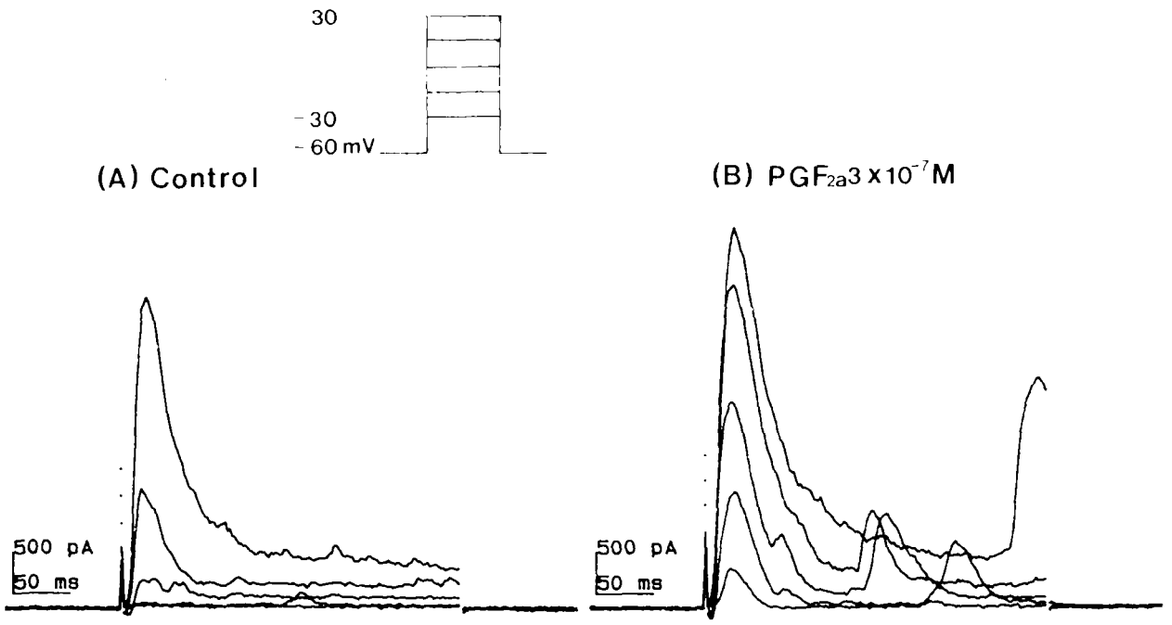


Fig. 17. The effect of $PGF_2\alpha$ on the calcium-dependent potassium outward currents. Transient and oscillatory outward currents were elicited by the depolarizing pulses to -30, -15, 0, 15, 30 mV from the holding potential of -60 mV, using a high K^+ solution containing 0.1 mM of EGTA in the pipette. These outward currents were enhanced by $PGF_2\alpha$ (0.3 μM) both in amplitude and frequency.

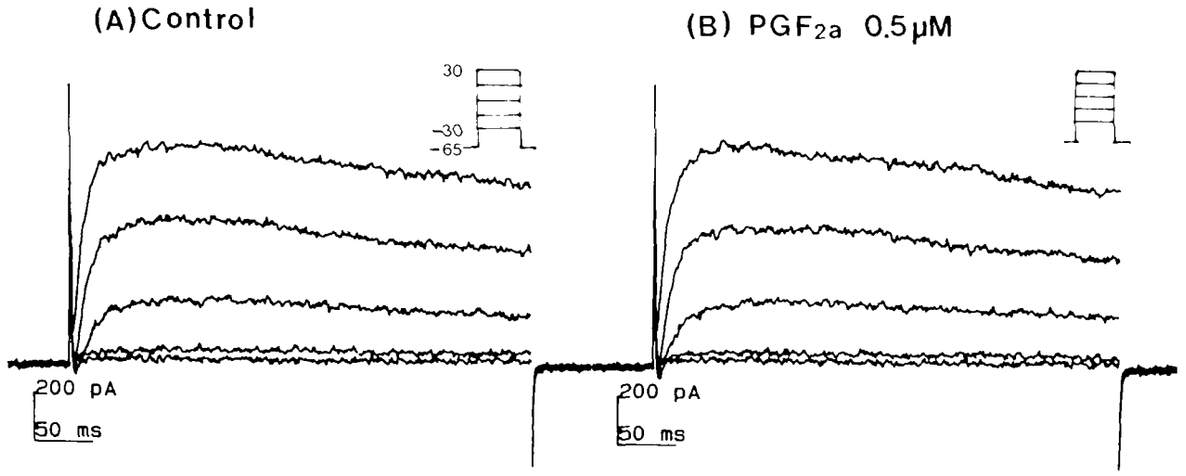


Fig. 18. The effect of $PGF_2\alpha$ on the voltage dependent delayed outward potassium current. The voltage-dependent sustained outward currents were elicited by the depolarizing pulses to -30, -15, 0, 15, 30 mV from the holding potential of -65 mV,using a high K^+ solution containing 10 mM of EGTA in the pipette. These currents were not changed by the application of $PGF_2\alpha$ (0.5 μM).

tetrakisphophate (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 $[Ca^{2+}]_i$). 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 IICR 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 IICR 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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