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Effects of Prostaglandin E_2 on the Spontaneous Contractions and Electrical Activities of the Antral Circular Muscle in Guinea-pig Stomach

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The spontaneous contractions of gastric smooth muscles are regulated by slow waves, which are modulated by both nervous system and humoral agents. This study was designed to examine the effects of prostaglandin E_2 (PGE_2) on the contractile and electrical activities of antral smooth muscles in guinea-pig stomach, using an intracellular recording technique. To elucidate the underlying mechanism for its effect on contractility, ionic currents were also measured using a whole-cell patch clamp method. The basal tone by PGE_2 was variable, whereas the magnitude of phasic contractions was reduced ($19.0 \pm 2.1\%$, n=19). The resting membrane potentials were hyperpolarized (-4.4 ± 0.5 mV, n=10), and plateau potentials were lowered (-2.9 ± 0.5 mV, n=10). In most cases, however, the initial peak potentials of slow waves were depolarized more by PGE_2 than those of control. The frequency of the slows wave was increased from 5.7 ± 0.2 cycles/min to 6.5 ± 0.2 (n=22). Voltage-operated Ca^{2+} currents were decreased by PGE_2 (n=5). Voltage-operated K^+ currents, both Ca-dependent and Ca-independent, were increased (n=5). These results suggest that PGE_2 plays an important role in the modulation of gastric smooth muscle activities, and its inhibitory effects on the contractility and activities of slow waves are resulted from both decrease of Ca^{2+} currents and increase of K^+ currents.

Key Words: PGE_2, Gastric smooth muscle, Slow waves, K^+ current, Ca^{2+} current

INTRODUCTION

The spontaneous contractions of gastrointestinal smooth muscles are regulated by myogenic oscillatory electrical activities called slow waves. The smooth muscle of guinea-pig stomach shows a marked regional difference in terms of electrical and mechanical properties (Kuriyama et al, 1975). The shape and frequency of slow waves are modulated by extrinsic and/or intrinsic nervous system. In addition, many humoral agents released in an endocrine and/or paracrine manner affect the electrical activities of the gastrointestinal smooth muscle. Metabolism of arachidonate produces several prostaglandins (PGs) in gastric smooth muscle and mucosa. The PGs constitute a group of naturally occurring C-20 unsaturated hydroxy fatty acids, of which the E (PGE) and F (PGF) series are known to be widely distributed in biologic tissues (Coleman et al, 1990). Naturally occurring PGEs inhibit gastric acid secretion in man (Classen et al, 1971) and inhibit ulcer formation in rat (Robert et al, 1968). Though many reports suggest that PGs play a physiological role in regulating electrical and mechanical behaviours of gastrointestinal smooth muscles (Bennet et al, 1968a, 1968b; Main & Whittle, 1975; Mishima & Kuriyama, 1976; Sanders, 1978; Sanders & Szurzewski, 1981; Kim et al, 1985), their

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results were different, depending on the tissues they used. The effects of PGs on ionic currents in gastrointestinal smooth muscle have not been reported yet. In vascular smooth muscle of rat tail artery, where PGE_2 evoked a sustained contractile response, PGE_2 inhibited the outward K current (Ren et al, 1995, 1996). Though rat aortic strips were relaxed by PGE_2 as observed in this experiment, cellular mechanisms could not be explained clearly due to the conflicting results; intracellular Ca^{2+} concentration was increased, Ca-dependent K currents were activated, and voltage-activated Ca^{2+} currents were inhibited (Serebryakov et al, 1994).

Previously, we reported the effects of $PGF_{2\alpha}$ on slow waves and ionic currents recorded in guinea-pig gastric myocytes (Kim et al, 1993). In that report, we suggested that $PGF_{2\alpha}$ acts as the dominant endogenous prostaglandins in circular muscle layer of guinea-pig antrum and presented potentiating effects of $PGF_{2\alpha}$ on slow waves and voltage-operated Ca currents. This study was designed to perform similar experiments to see the effects of PGE_2, another important prostaglandin, on slow waves and ionic currents in guinea-pig stomach. In guinea-pig pyloric circular muscle, Mishima and Kuriyama (1976) have reported the PGE_1-induced hyperpolarization of membrane potential and inhibition of the slow wave generation, resulting in the inhibition of mechanical activity. In canine antrum, PGE_2 also produced hyperpolairzation and inhibition of mechanical contractions (Sanders et al, 1983). Since the hyperpolarization was accompanied by the decrease in membrane resistance, it was suggested that such hyperpolarizing action was due to the increase of the ionic conductance in the smooth muscle cell membrane.

In this experiment we report the similar hyperpolarizing effects of PGE_2 on membrane potential of the antral circular smooth muscle in guinea-pig stomach, and its effects on voltage-operated ionic currents.

METHODS

Cell isolation

Guinea-pigs of either sex weighing 300~350 g were exsanguinated after stunning. The stomach was isolated and cut in the longitudinal direction along the lesser curvature in phosphate-buffered Tyrode solution. The antral part of stomach was cut, and the mucosal layer was separated from the muscle layers. The circular muscle layer was dissected from the longitudinal layer using fine scissors and made into small segments (2×3 mm). These segments were incubated in a medium modified from the Kraft-Brühe (K-B) medium (Isenberg & Klckner, 1982) for 30 min at 4°C and were transferred into nominal Ca^{2+} -free physiological salt solution (PSS) containing 0.1% collagenase (Böhringer Mannheim or Wako), 0.05% dithiothreitol, 0.1% trypsin inhibitor and 0.2% bovine serum albumin, and then incubated for 15~25 min at 35°C. After digestion, the supernatant was discarded and the remaining softened muscle segments were transferred again into the modified K-B medium and single cells were dispersed by gentle agitation with a wide-bored glass pipette. Isolated gastric myocytes were kept in the modified K-B medium at 4°C until use. All experiments were carried out within 12 hours of harvesting cells and performed at room temperature.

The measurement of membrane currents

Isolated cells were transferred to a small chamber (400 μl) on the stage of an inverted microscope (IMT-2, Olympus, Japan). The chamber was perfused with physiological salt solution (PSS, 2~3 ml/min). Glass pipettes with a resistance of 2~4 MΩ were used to make a gigaseal of 5~10 GΩ. Standard whole-cell patch clamp techniques were used (Hamill et al, 1981).

An Axopatch-1C patch-clamp amplifier (Axon instruments, Burlingame, USA) was used to record membrane currents and command pulses were applied using an IBM-compatible AT computer and pCLAMP software v.5.51 (Axon Instrument, Burlingame, USA). The data were filtered at 5 kHz and displayed on a digital oscilloscope (PM 3350, Phillips, Netherlands) and the computer monitor, and recorded in a pen recorder (Recorder 220, Gould, Cleveland, USA).

Measurement of isometric contractions and intracellular recording of the electrical activity

Muscle strips (2~3 mm wide, 10~12 mm long) from the proximal part of the antrum were cut parallel to the circular fibers, and mounted on a silicon rubber in a 2 ml horizontal chamber. The strip was pinned out at one end with tiny pins and the other end was connected to a force transducer to record the

isometric contractions. The strip was constantly perfused at a rate of 2~3 ml/min with CO_2/bicarbonate-buffered Tyrode solution. Electrical activities were recorded using the conventional intracellular recording technique and drawn by a chart recorder (MX-6, Device Ltd, Britain). Only glass microelectrodes with tip resistance of 40~80 MΩ were used by filling them with 3 M KCl.

Solutions

Phosphate-buffered Tyrode solution contained (in mM) NaCl 147, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.8, NaH_2PO_4 0.42, Na_2HPO_4 1.81, glucose 5.5, pH 7.3. CO_2/bicarbonate-buffered Tyrode solution contained (in mM) NaCl 116, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1, $NaHCO_3$ 24, glucose 5 (pH 7.3~7.4, bubbled with 5% CO_2-95% O_2). Ca^{2+}-free PSS contained (in mM) NaCl 135, KCl 5, $MgCl_2$ 1, glucose 5, HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 5, and the pH was adjusted to 7.3 by Tris. PSS contained 1.8 mM $CaCl_2$ in the Ca^{2+}-free PSS. Modified K-B solution contained (in mM) L-glutamate 50, KCl 50, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA (ethyleneglycol-bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid) 0.5 and pH was adjusted to 7.3 by KOH. For the recording of voltage-dependent K^+ current, we used pipette solution containing (in mM) K-aspartate 100, MgATP 5, di-tris-creatine phosphate 5, KCl 20, $MgCl_2$ 1, EGTA 10, HEPES 5 and pH was adjusted to 7.3 by KOH. For the recording of Ca^{2+} -activated K^+ current only EGTA concentration was lowered to 0.1 mM. All the potassiums in the pipette solution were replaced with Cs and TEA (20 mM) in order to block the K current for the recording of voltage-operated Ca^{2+} current. All drugs used in this experiments were purchased from Sigma.

Statistics

All values are expressed as means$\pm$*SE*. Statistical analysis was performed using the Student's *t* test. Differences were considered significant when P value was less than 0.05.

RESULTS

Effects of PGE_2 on the isometric contractions and slow waves

PGE_2 (0.3 μM) induced inhibitory effects on the magnitudes of phasic contractions (19.0$\pm$2.1%, n=19). But basal tones did not showed consistent changes in pattern; they increased in 3 out of 19 strips, not changed in 6 strips, and decreased in 8 strips (Fig. 1). In most cases (15 out of 19 experiments), transient increase of the phasic contractions and basal tone preceded the overall sustained inhibitory effects. Those inhibitory effects were completely reversible and were not affected by the pretreatment with atropine, guanethidine, and tetrodotoxin, indicating that PGE_2 acted directly on smooth muscle cells (data not shown).

Conventional intracellular recordings were used to see the effects of PGE_2 on the electrical activity (Fig. 2 & Fig. 3). Values of usual resting membrane potentials were around -60 mV. When PGE_2 was administered in the bath solution, resting membrane potentials were hyperpolarized by an average of -4.4 ± 0.53 mV, and membrane potentials at plateau phase

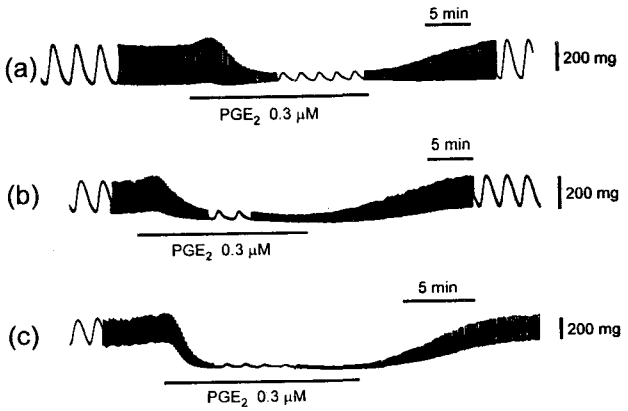

Fig. 1. Effects of PGE_2 on the contractility of antral circular smooth muscles in guinea-pig stomach. Magnitudes of both spontaneous phasic contractions and basal tones were reduced by 0.3 μM of PGE_2. The degree of reduction was different in 3 strips; minimum in trace (a), moderate in trace (b), and severe in trace (c). In cases of (a) and (b), transient increase of the amplitude of spontaneous contractions preceded the sustained inhibitory effects of PGE_2. Recording speed was increased intermittently for a while to make it easier to compare phasic contractions.

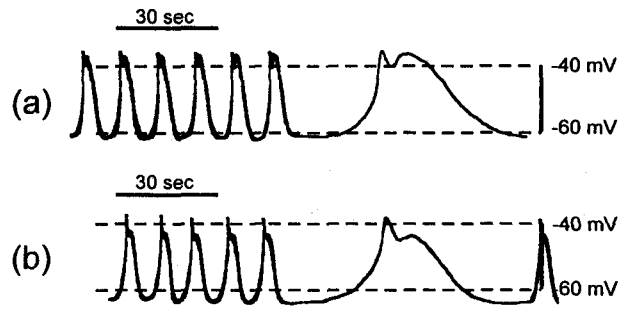

Fig. 2. Typical effects of PGE_2 on the resting membrane potential and the slow waves of circular muscle. Conventional intracellular glass-microelectrode technique was used to record the membrane potential of circular muscle. Trace (a) and (b) were recorded in the same cell before (a) and during (b) the treatment with PGE_2. 0.3 μM of PGE_2 induced a prominent hyperpolarization of the resting membrane potential. The initial peak and plateau potentials of slow waves were hyperpolarized, too.

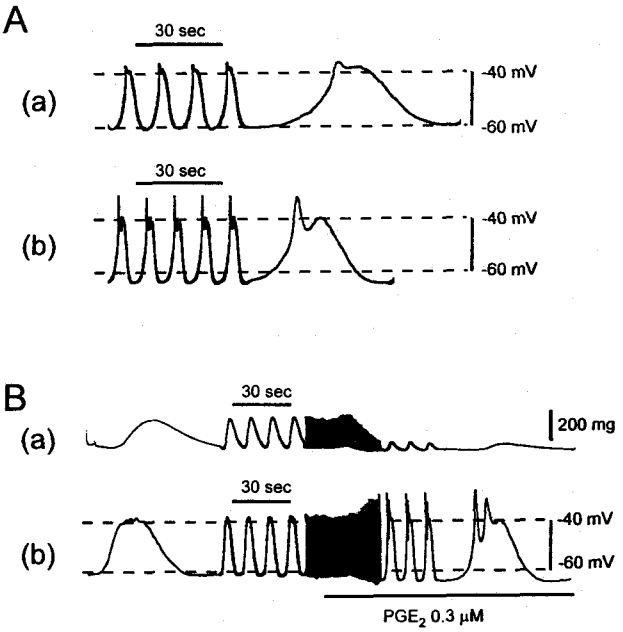

Fig. 3. Effects of PGE_2 on the resting membrane potential and the slow waves recorded in other strips. A: 0.3 μM of PGE_2 induced hyperpolarization of the resting membrane potential and plateau potential, which is similar to the results shown in Fig. 2. Howerer, the peak potential of the slow wave was depolarized more by PGE_2 (b). B: The contractile (a) and electrical (b) responses were recorded simultaneously. This trace shows the similar result to trace A with prominent depolarizations of the initial peak potential, which cannot be correlated with mechanical contractions. Note that contractions begin to decrease even before the initial peak potentials were changed, that is, the size of slow waves was increased.

were lowered by an average of -2.9 ± 0.52 (n=10). Thus, slow waves were increased by PGE_2 in classic terms of the magnitude. The initial peak potentials were depolarized more by PGE_2. This can be interpreted as meaning that the initial peak potential contributes little to the magnitude of the contractile response. However, they might contribute more to the frequency response as slow wave frequency was increased from 5.7 ± 0.2 cycles/min to 6.5 ± 0.2 by PGE_2 (n=10).

Effects of PGE_2 on voltage-operated Ca current

Single gastric myocytes were voltage-clamped in whole-cell configuration. High Cs pipette solution was adopted to block the outward K currents. Membrane potential was held at -80 mV and stepped to various levels (from -50 mV to $+50$ mV). As shown in our previous report (Kim et al, 1993), Ca inward current was activated by depolarizing pulses, and this current was composed of L-type Ca currents (high voltage-activated Ca current).

In 5 out of 8 cells, the peak amplitudes of inward currents were decreased by PGE_2 (Fig. 4A). For those 5 cells, peak amplitudes were normalized, averaged and plotted according to the test voltages (Fig. 4B).

Effects of PGE_2 on K currents

High K pipette solution containing 10 mM EGTA was used to record voltage-dependent, Ca-independent K currents. Membrane potential was held at -60 mV and stepped to various depolarized levels. Outward currents showed initial rapid activation and then slow inactivation during the pulse duration (Fig. 5A-(a)). These outward K currents were increased by bath applied PGE_2 (Fig. 5A-(b)). The difference currents revealed that the later inactivating component was affected more; this component was maintained or even increased progressively (Fig. 5A-(c), 5B). Effects of PGE_2 on K outward currents were also prominent when the concentration of EGTA was lowered to 0.1 mM (Fig. 6) so that mainly Ca-activated K currents could be recorded. As shown in Fig. 6, the larger and noisier outward currents, which are typical of Ca-activated K currents, showed the same tendency as

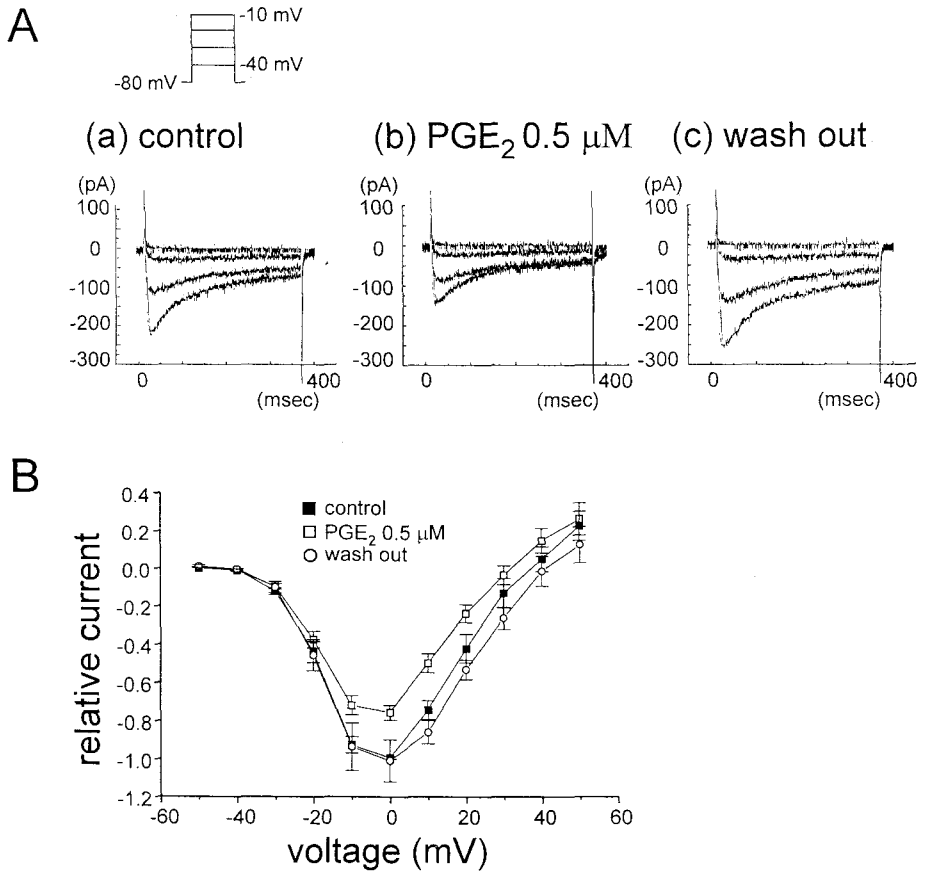

Fig. 4. Effects of PGE_2 on the voltage-operated Ca current. In the whole-cell configuration, membrane potential was held at −80 mV and depolarized to various voltage levels for 350 ms. A: Only 4 traces (steps from −40 mV to −10 mV by 10 mV increment) are shown and the reduction of the Ca currents is obvious. B: All the peak currents were normalized against the peak value obtained at 0 mV and were averaged and plotted. At 0 mV relative current was decreased by PGE_2 (76.0±4.0%, n=5) and was recovered by wash-out of bath solution (96.0±5.1%).

voltage-dependent K currents, that is, the time-dependent decaying process was attenuated more prominently.

DISCUSSION

The results obtained from this experiment can be summarized as follows: 1) PGE_2 influences an inhibitory effect on the contractile activities, 2) PGE_2 induces hyperpolarization of membrane potential in the plateau and the base, while induces depolarization of initial peak potential of slow waves, 3) PGE_2 increases the frequency of slow waves, and 4) PGE_2 increases the amplitudes of K outward currents, while moderately decreases those of Ca inward currents.

To our knowledge, this is the first report on the direct effects of PGE_2 on ionic currents of gastro-intestinal smooth muscle cells recorded using a whole-cell patch clamp method. It has been reported that PGE_2 produced a reduction of Ca currents in sympathetic neurons (Ikeda, 1992). Our data showed that PGE_2 increased the K outward currents in guinea-pig antrum (Fig. 5 & Fig. 6). It is interesting that PGE_2 increased the outward currents to a larger extent at the end of each step of depolarization. This means that PGE_2 attenuated the inactivating process of the outward currents. Such characteristics of PGE_2-sensitive outward currents cannot be ascribed to any single category of channels. It has been reported that there are more than two kinds of K currents in guinea-pig antral myocyte; Ca-dependent one and Ca-independent one (Noack et al, 1992). In general, the voltage-activated, Ca-independent K currents usually

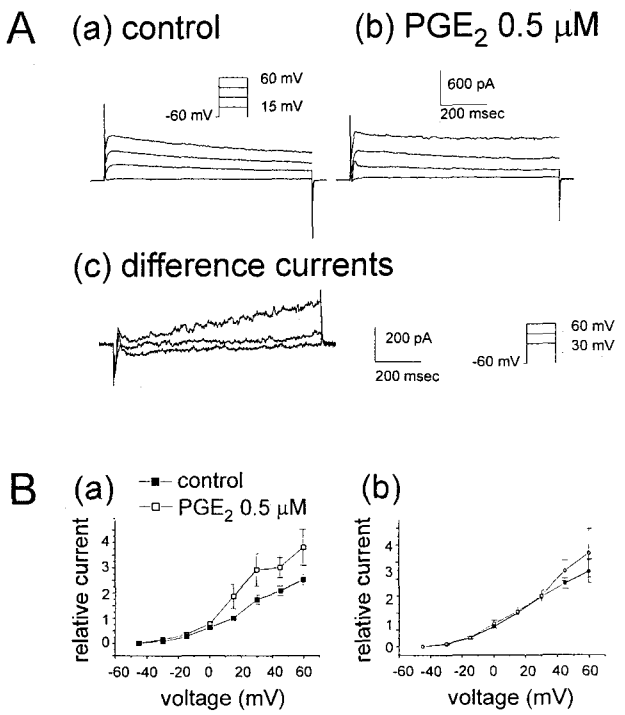

Fig. 5. Effects of PGE_2 on the voltage-operated K current. Ca-independent and Voltage-operated K currents were recorded using high EGTA (10 mM), high K solution in the pipette for 800 ms. A: 0.5 μM of PGE_2 influenced later sustained component more prominently (b). For more detailed explanation, refer to the result in the text. B: Findings in trace A can be clearly seen in averaged plot, where currents were normalized against the steady-state value at +15 mV (n=5). Steady-state values (a) were increased above the range of 0 mV, but peak values (b) showed no difference statistically in the whole range up to 60 mV.

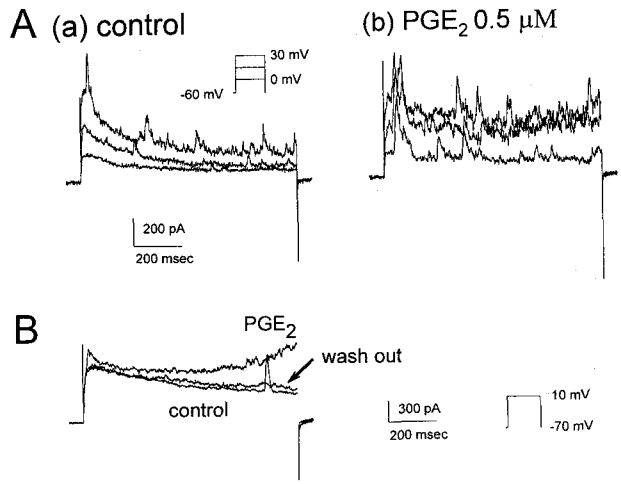

Fig. 6. Effects of PGE_2 on the Ca-dependent K current. K currents were recorded using low EGTA (0.1 mM), high K solution in the pipette. A: Noisy outward currents with spontaneous oscillations, typical of large conductance Ca-activated K currents, were observed in this condition. These currents were increased by PGE_2 (b) in the same manner as Ca-independent, voltage-operated K currents. Increase of the later steady-state component was more prominent, too. B: In other cell the difference currents were recorded at step potential of 10 mV. The same result can be seen more clearly.

show a time-dependent inactivating property. PGE_2-induced changes might have hindered this inactivating process. However, the fact that the changes of outward current in Fig. 6 (when the concentration of EGTA, the Ca-buffering agent, was lowered to 0.1 mM) were more prominent than those in Fig. 5 indicates that the Ca-activated K channels are also likely to be the target for the modulation by PGE_2. In smooth muscle cells, Ca-activated K channels with large conductance (BK channels or maxi-K channels) have been widely found and reported to be the target of modulation through phosphorylation by proeitn kinase A (Kume et al, 1989; Carl et al, 1991) or the unknown membrane-delimited kinase (Lee et al, 1994). PGE_2 is known to induce increase in the concentration of cAMP in smooth muscle cells through its own receptors (Coleman et al, 1990), and part of the effects might be related with the increased level of cAMP in the cytoplasm. A significant hyperpolarization of gastric smooth muscle was observed when forskolin or isoprenaline was applied, which can increase the cytoplasmic level of cAMP and activate protein kinase A (unpulished observation). Although the exact class of K channels modulated by PGE_2 is not clear yet, such an increase in membrane conductance to outward currents coincides well with the result reported by Mishima & Kuriyama (1976), where they observed an increase of membrane conductance and hyperpolarization by PGE_1 and PGE_2 in guinea-pig gastric muscle. So, the PGE_2-induced hyperpolarization of resting membrane potential and plateau potential seems to be closely related to the increase of K conductance.

PGE_2 played dual effects on slow waves in guinea -pig gastric smooth muscle; the hyperpolarization, and the potentiation of upstroke-depolarization. The results shown in Fig. 2 and Fig. 3 indicate that PGE_2 decreases the amplitudes of spontaneous contractions by suppressing the degree of the plateau depolarization of slow waves. It is generally known that the

phasic oscillatory influx of Ca through voltage-activated channels in depolarization triggers the spontaneous contraction of visceral smooth muscle. It has been reported that the height of plateau is the major factor in electromechanical coupling in the antrum (Morgan & Szurzewski, 1979). Actually, those spontaneous contractions are abolished by organic Ca channel blockers and heavy metal ions, which can block the Ca channels in smooth muscle cells. As shown in Fig. 4B, the activating threshold voltage of the Ca channel is around −40 mV. So, the duration and extent of depolarization of each slow wave above this 'threshold voltage (−40 mV)' will determine the amount of Ca-influx which will trigger each phasic contraction. Changes in the plateau phase in Fig. 2 and Fig. 3 clearly show such relationship between the extent of plateau-depolarization and the magnitude of phasic contractions. However, it was not easy to correlate the size of slow wave above the 'threshold voltage (−40 mV)' with the size of spontaneous contraction in some cells (Fig. 3B). In such a case, the accentuation of slow wave was so strong that the initial peak potential of the slow wave was depolarized much more over the threshold. This means that the amplitude of slow wave was increased. One possible explanation is that the initial peak potential contributes primarily to the frequency response, while the later plateau potential contributes to the size response. Therefore, the frequency of mechanical contractions and slow waves was increased, while the size was decreased by PGE_2. The other interpretation is that PGE_2 affected contractile apparatus directly and/or via second messenger cascade mechanism. The effects of PGE_2 on voltage-operated Ca currents are so complicated that it is difficult to translate them into changes in slow waves. A moderate inhibitory effect on inward Ca currents (Fig. 4) can explain the suppression of plateau-depolarization to some extent, although it cannot be correlated with the potentiation of upstroke depolarization. Before any attempts are made to explain these results, it should be noted that the muscle preparations used in this experiment are composed of muscle cells of circular layer as well as enteric nerve cells and myocytes of longitudinal layer. In GI smooth muscle, PGs of the E series are known to have different effects on mechanical contractility depending upon the muscle layer; stimulatory and inhibitory effects on longitudinal and circular layer, respectively (Bennet et al, 1968 a; Bennet et al, 1968b; Mishima & Kuriyama, 1976; Milenov et al, 1980). As the muscle strips used in this experiment were not dissected into the isolated circular layer, there is a possibility that effects of PGE_2 on the smooth muscle cells in longitudinal layer might have influenced the electrical activity recorded through the microelectrode impaled into the cells in circular muscle layer. In addition, the transient enhancement of contractile activity shown at the initial phase of PGE_2-treatment (Fig. 1) might be an artifact due to the positive inotropic effect of PGE_2 on the myocytes of longitudinal layer. Also, we cannot completely exclude a possibility that PGE_2 has exerted influence through the modulation of enteric nerve cell.

Accelerating effects of PGE_2 on slow wave frequencies have been reported in canine antrum (Sanders et al, 1983) and feline antrum (Kim et al, 1985). In canine antrum, mechanical contraction was significantly inhibited by PGE_2, while in feline antrum the resting tension was not significantly influenced. Sanders et al (1983) suggested that the refractory period between slow waves is decreased by PGE_2, and such phenomenon might have been simply due to the decrease in slow wave duration caused by PGE_2. In guinea-pig antrum, PGE_2 also induced statistically small but significant increase in slow wave frequencies. This calls for further studies on PGE_2 roles.

Prostaglandins, like other local hormones, produce their effects by interacting with specific receptors on cell membrane. It is suggested that distinct receptors exist for each of the five naturally occurring prostanoids, PGD, PGE_2, $PGF_{2\alpha}$, PGI_2 and TxA_2 (DP, EP, FP, IP & TP, respectively), and receptors for PGE_2 can be subdivided into three groups EP_1, EP_2 and EP_3, where the activation of EP_2 relaxes smooth muscle, and that of EP_1 or EP_3 induces contraction (Coleman et al, 1990). As there are possibilities that the activation of different subgroups of PGE_2 receptors can induce different results in intracellular signalling process, further studies on the effects of PGE_2 should be focused upon the specific actions of receptor subgroups of smooth muscle cells.

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