EFFECTS OF PROSTAGLANDIN E₂ ON THE ELECTRICAL PROPERTY OF THE PREGNANT MOUSE MYOMETRIUM

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Abstract At any stage of gestation, low concentrations of PGE₂ increased the frequency and number of spikes in a train discharge without any marked change of the membrane potential. An increased concentration depolarized the membrane and produced continuous spike generation. A further increase in concentration of PGE₂ produced a depolarization block of spike generation. The sensitivity of the myometrium to PGE₂ was markedly increased during the progress of gestation so that at the late stage of gestation, the sensitivity was more than 100 times greater than at the early stage. The longitudinal muscle showed a higher sensitivity to PGE₂ than the circular muscle. However, both muscles showed a similar sensitivity to oxytocin. In the longitudinal muscle, desensitization to PGE₂ occurred more quickly than to oxytocin. When PGE₂ produced a slight depolarization of the membrane, the membrane resistance was increased. However, when the membrane potential was displaced to the control level by applying a hyperpolarizing current, the membrane resistance was slightly reduced. Displacement of the membrane potential to the level before drug application restored spike generation. However, the membrane resistance was still reduced. In Na-deficient Locke solution or in Ca-free Mg-Locke solution, the depolarization induced by PGE₂ was suppressed but not suppressed by, in K-deficient Locke solution.

Prostaglandins (E and F series) are known to be distributed widely in biological tissues. The biological actions of prostaglandins (PG) which differ from one series to another vary not only according to the tissues and species, but also with the hormonal state of the tissues. For example, in the human myometrium, the frequency and amplitude of the spontaneous contractions are usually suppressed by PGE, but are increased by PGF; on the other hand, the myometria of guinea

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pig, rat, and rabbit contract in response to both PGE and PGF (Bergström et al., 1959; Eliasson, 1959; Horton and Main, 1963; Sullivan, 1966; Paton and Daniel, 1967). The human nonpregnant myometrium is several times more sensitive to the relaxation effect of PGE at the time of ovulation, but is more sensitive to PGF compounds during gestation. In the guinea pig and rat, progesterone added in vitro depressed the sensitivity of PGE and PGF (see reviews of Bergström et al., 1968; Horton, 1969).

Recently Osa et al. (1974) observed the effects of synthetic PGE₂ on the electrical and mechanical activities of the pregnant mouse myometrium and they came to the conclusion that the excitatory action of PGE₂ varied with the ratio of [Ca]₀ to [Na]₀.

The present experiments were to investigate further the effects of PGE₂ on the electrical activity of the smooth muscle cell membrane of the pregnant mouse myometrium. The roles of PGE₂ on the mouse myometrium in relation to the ionic permeability of the membrane during gestation are discussed.

METHODS

Pregnant mice (dd system) were stunned and bled. The uteri were excised and either longitudinal or circular strips were prepared. The period of gestation was calculated from the single day that the male mice were caged with the female mice.

To record the mechanical activity alone, a chamber of small capacity (0.4 ml) was used. Strips 1 mm in width and 5 mm in length were weakly stretched within this chamber. To record the electrical activity, the partition stimulation method was used (Abe and Tomita, 1968).

Ionic compositions of the normal bathing solution were the same as described by Osa (1974). To prepare Na-deficient Locke solution, NaCl was replaced with either isosmolar sucrose or Tris. To prepare Ca-free Locke solution, MgCl₂ (4.4 mM twice the normal concentration of CaCl₂) was added to the solution instead of CaCl₂. K-deficient Locke solution was prepared by a reduction of KCl to one-tenth of the normal concentration. To maintain the osmolarity, an additional 5 mM NaCl was added to the K-deficient solution. Excess K-Locke solution was prepared by reduction of equimolar Na ion to maintain the osmolarity.

The bathing media were kept at 35°C and perfused at a constant rate (1 ml/sec).

The drugs used were synthetic prostaglandin E₂ (PG 502; Ono Pharm. Co., Ltd.) and oxytocin (Atonin-O; Teikoku Pharm. Co., Ltd.). The concentrations of PGE₂ are given in g/ml and those of oxytocin in U/ml.

RESULTS

The effects of PGE₂ on the electrical activity of the myometrium during gestation
and in comparison with the effects of oxytocin

PGE₂ showed an excitatory action on the mouse myometrium. In low concentrations of PGE₂, the spike frequency, the number of spikes in the train discharge and the number of train discharges were increased. However, the actual concentration of PGE₂ to produce excitation varied markedly during the progress of gestation. Membrane activity induced by PGE₂ was classified tentatively into three grades in the following way: First grade, which increased only the spike frequency, the number of spikes in a train discharge and the frequency of train discharges without any marked change of the membrane potential; Second grade, which depolarized the membrane and induced continuous generation of spikes eliminating the quiescent periods between train discharges, and Third grade, whose largest effect was the depolarization block of spike generation.

Figure 1 shows the effects of PGE₂ on the membrane activity at various stages of the gestation. During both the early and middle stages of gestation, the first grade response and depolarization block of the spike activity appeared at concentrations of $10^{-8}$ and $10^{-5}$ g/ml, respectively. However, during the late stage of gestation (after the 14th day of gestation), the responses of the membrane appeared at concentrations below $10^{-9}$ and $10^{-8}$ g/ml, respectively.

The spike frequency and patterns of the spontaneously generated train discharge vary not only with the stage of gestation, but also with the time in the organ bath and the degree of stretch (Kuriyama, 1964; Casteels and Kuriyama, 1965). Therefore, the above concentrations are only relative, although the sensitivity of the muscle membrane to PGE₂ increased at least one hundred times at the late stage of gestation compared with the early stage.
Figure 2 shows the effects of various concentrations of PGE$_2$ and oxytocin on the membrane activity of the longitudinal muscle of the myometrium (17th day of gestation). The third grade response of the membrane appeared at a concentration of $10^{-6}$ g/ml and similar responses could be produced by $10^{-3}$ U/ml oxytocin. The patterns of the membrane responses evoked by PGE$_2$ and by oxytocin were nearly the same. Marked differences in the effects of PGE$_2$ and oxytocin on the circular muscle of the myometrium, however, were observed. As reported by Osa (1974), a difference in shape of the train discharges recorded from the longitudinal and circular muscle could be distinguished. Train discharges in the longitudinal muscle appeared as burst discharges on a plateau-like depolarization. On the other hand, in the circular muscle the amplitude of this depolarization was much larger and the spike superimposed on it was often abortive.

Fig. 2. Effects of PGE$_2$ or oxytocin on the electrical activity of the longitudinal muscle (myometrium on 17th day of pregnancy at the concentrations shown below each trace). Bars in the figure indicate the presence of PGE$_2$ or oxytocin.

Figure 3 shows the effects of PGE$_2$ and oxytocin on the circular muscle cell membrane of the myometrium (18th day of gestation). The microelectrode was inserted deeply into the circular muscle from the serosal side. Only $10^{-5}$ g/ml PGE$_2$ was necessary to produce the second grade effect on membrane activity. On the other hand, oxytocin ($10^{-3}$ U/ml) caused a depolarization block of the membrane activity of the circular muscle as observed in the longitudinal muscle cell membrane.

The low sensitivity of the circular muscle to PGE$_2$ in comparison with that of
Fig. 3. Effects of PGE₂ or oxytocin on the electrical activity of the circular muscle (myometrium on 18th day of pregnancy at the concentrations shown below each trace). Bars in the figure indicate presence of the drug.

The longitudinal muscle could also be seen in its mechanical response. Spontaneous mechanical activity was lower in the circular muscle. With the application of PGE₂ (10⁻⁷ g/ml) the mechanical activity of the longitudinal muscle increased in frequency and was superimposed on an increased muscle tone. However, in the circular muscle, only the frequency of the mechanical response was increased.

**The effects of PGE₂ on the membrane resistance**

To calculate the relative changes in membrane resistance before and during depolarization of the membrane from the amplitudes of the electrotonic potential, the microelectrode was inserted within 0.3 mm from the stimulating partition and inward current pulses were applied to the preparation. Since the myometrium possesses a cable-like property for the spread of the electrical current, changes in the relative membrane resistance can be expressed as follows:

\[
\left( \frac{V'}{V} \right)^2 = \frac{r_m r_i}{r_i} \left( \frac{\exp^{-\frac{\xi}{\lambda}}}{\exp^{-\frac{\xi'}{\lambda}}} \right)^2
\]

(HODGKIN and RUSHTON, 1946),

where \( V \) is the steady state electrotonic potential, \( r_m \) is the membrane resistance, \( \xi \) is the distance between recording and stimulating electrodes, \( r_i \) is the internal longitudinal resistance, \( \lambda \) is the length constant, and the symbol " ' " indicates the presence of PGE₂, excess K or electrical displacement of the membrane potential. Provided \( r_i \) is to remain the same before and after application of the drug.
and \( x \) (less than 0.3 mm) is much smaller than (1.2 mm), the above equation can be simplified to \( \left( V'/V \right)^2 \simeq r_m'/r_m \).

However, if the drug markedly decreases the length constant, the obtained value from the above equation is not reliable even though the recording micro-electrode is inserted very close to the stimulating electrode.

Figure 4 shows the effects of PGE\(_2\) on the membrane potential and membrane resistance of the longitudinal muscle of the myometrium (14th day of gestation). When \( 10^{-7} \) g/ml PGE\(_2\) was applied to the tissue, the membrane was depolarized and increased the frequency of spike discharge (second grade effect). The amplitudes of electrotonic potentials produced by an application of inward current pulses were larger during PGE\(_2\) treatment than before application of the drug (a). An application of outward current pulses evoked spikes and the amplitude of the electrotonic potential was smaller in proportion to depolarization of the membrane (c). If the membrane potential was displaced during the application of PGE\(_2\) to the level before the treatment with the drug, the amplitude of the electrotonic

![Fig. 4. Effects of PGE\(_2\) on the membrane potential and the amplitude of electrotonic potential. a-e, 14th day of gestation; f-g, 18th day of gestation; a, b, f and g, inward current pulses were applied; c, d and e, outward current pulses were applied. Interval between d and e was 30 sec.](image-url)
potentials produced by inward current pulses was slightly less than the value obtained before the application of the drug.

During the generation of the third grade response, the application of inward currents of various intensities shifted the membrane potential level towards the original level and spikes reappeared. The amplitude of the spikes depended on the magnitude of the displacement of the membrane potential. When the membrane potential was displaced to a level close to that before the application of the drug, the amplitude of the spike often became larger than that in the control Locke solution. Therefore, the depolarization block of the spike was caused by inactivation of the spike-generating mechanism.

Figure 5 shows an example of current-voltage relationships recorded before and during the application of PGE_2. The membrane potential was displaced in either a depolarized or hyperpolarized direction in steps; short inward current pulses of different intensities were applied. When the current-voltage relationships were compared in the two different conditions, the amplitude of the electrotonic potential in the presence of PGE_2 was consistently lower than that observed in the absence of the drug at the same membrane potential level.

To compare with the effects of PGE_2, the effects of excess K-Locke solution on the membrane potential and membrane resistance were observed (Fig. 6). When K ion concentration was increased at any given concentration, the mem-
brane was consistently depolarized. However, the amplitudes of the electrotonic potentials were not proportionally reduced. For example, on slight depolarization of the membrane induced by twice the normal K concentration, the amplitude of

![Graph of Longitudinal muscle (19th day)](image)

Fig. 6. Effects of various concentrations of K ion on the membrane potential and the amplitude of the electrotonic potential (18th day of gestation). Long bars under the electrical records indicate periods of excess $[K]_0$ application. Inward current pulses were applied throughout the experiment.

![Graph of Membrane Resistance](image)

Fig. 7. Effects of displacements of the membrane potential on the membrane resistance (18th day of gestation). Membrane potential was displaced by PGE2 (○), by excess $[K]_0$ or by current injection (×). Ordinate, Relative change in the membrane resistance expressed as $(V'/V)^2$ by simplification of the cable equation (see text); Abscissa, Membrane potential level ($-mV$).
the electrotonic potential was larger than that recorded in Locke solution; further increase in K concentration induced further depolarization of the membrane, but reduced the amplitude of the electrotonic potential.

Figure 7 shows the relationship between relative change of the membrane resistance and the membrane potential before and during the application of PGE₂, excess K and electrical displacement of the membrane potential. Even though the membrane potential was displaced with three different procedures, small depolarization of the membrane consistently increased the resistance and marked depolarization reduced the membrane resistance. Measurements of the amplitudes of the electrotonic potential were not accurate especially in the presence of excess K, since the membrane was always active and the changes in the membrane resistance would cause changes in the length constant. Nevertheless, these results suggest that increased membrane resistance during the slight depolarization of the membrane is not a specific action of PGE₂, but is a membrane property of mouse myometrium.

The effects of PGE₂ on the myometrium in various ionic environments

In K-deficient Locke solution, the membrane was transiently depolarized, then it gradually repolarized within 20 min to a level close to the resting membrane potential level. Although spikes appeared as continuous discharges as shown in Fig. 8 (a and b), the application of PGE₂ (10⁻⁶ g/ml) rapidly produced depolarization of the membrane and blocked spike generation (c). When the PGE₂ was rinsed out with K-deficient solution, the membrane gradually repolarized and spikes were again generated. However, the membrane potential and the spike amplitude were not completely restored to the values before the application of PGE₂. Repeated application of PGE₂ at intervals of 15 min caused depolarization and blocked spike generation. After the tissue was rinsed with the K-deficient solution, restoration of the membrane potential was less complete and the membrane activity became abortive (e). On washing the tissue with Locke solution, the membrane was at first markedly hyperpolarized (d and e), and the membrane again depolarized to the resting level over a period of more than 10 min (f). Spike generation depended on the membrane potential level. The hyperpolarization of the membrane was probably due to activation of an electrogenic Na pump (TAYLOR et al., 1970).

In Na-deficient Locke (sucrose or Tris) solution, depolarization of the membrane by PGE₂ was depressed. In sucrose Locke solution, the membrane was markedly depolarized (Fig. 9a). The depolarization lasted between 30-45 min, then the membrane potential gradually returned to a level close to the resting level. When the membrane potential returned to a level close to the resting level, PGE₂ evoked a small depolarization and an increase in spike frequency (b and c). When the tissue was perfused again with Locke solution, the membrane was at first markedly hyperpolarized (d and e), and the membrane again depolarized to the resting
membrane potential level (f). Similar results were observed in Tris-Locke solution to those in sucrose-Locke solution. However, the initial depolarization of the membrane in the presence of Tris-Locke solution was less well maintained during long exposure.

The effects of PGE$_2$ in Ca-free Mg Locke solution were studied. Mg ion may substitute for Ca ion to stabilize the membrane, but not for spike generation. Mg ion was, therefore, added to the Ca-free solution to suppress the depolarization of the membrane. Figure 10 shows the effects of PGE$_2$ on the longitudinal muscle of the myometrium (7th day of gestation) in Ca-free Mg-Locke solution. When Mg ions (4.4 mM) were added to a Ca-free Locke solution, the membrane was slightly hyperpolarized compared with the control, and spontaneous spike generation was blocked (b). Outward current pulses could evoke spikes even after 10 min of perfusion. When PGE$_2$ (10$^{-6}$ g/ml) was added to the tissue after 10 min
Fig 9. Effects of PGE₂ on the electrical activity of the myometrium (14th day of gestation) in Na-deficient (sucrose) Locke solution. a, effect of Na-deficient Locke solution; b, effect of 10⁻⁷g/ml PGE₂ after 60 min perfusion with Na-deficient Locke solution; c, effects of 10⁻⁷g/ml PGE₂; d, rinsed with Locke solution; e and f, recovery of membrane activity. Dots in the figure indicate applications of the drug and perfusing solution, respectively. b, c, d, e, and f are recorded after 60 min, 30 min, 30 min, 10 min and 3 min from the preceding record, respectively.

Of perfusion, the membrane was depolarized and spikes were transiently evoked which were nearly the same amplitude as in the control (c). However, spike generation soon stopped and the depolarization was smaller than in Locke solution (a). After 20 min of perfusion of the preparation in the Ca-free Mg Locke solution, PGE₂ still depolarized the membrane, but even outward current pulses of strong intensity could not evoke spikes (e).

The effects of carbachol and PGE₂ on the K-induced contracture were observed. Isotonic K-Locke solution produced a large contracture which gradually declined to a level close to the resting level, i.e., the tonic phase of the contracture was small. When the above agents were applied to the tissue during the tonic response, a small transient mechanical response could be evoked. The magnitudes of the mechanical response evoked by carbachol and PGE₂ were nearly the same. It is already known that carbachol releases sequestered Ca in the cell and also increases the influx of Ca ions in an isotonic K-solution (SCHILD, 1964).
Fig. 10. Effects of PGE₂ on the membrane activity of the longitudinal muscle of the pregnant mouse myometrium on the 7th day. a–d and e are observed from two different specimens, respectively. a, effect of 10⁻⁶ g/ml PGE₂ in Locke solution; b, effect of Ca-free Mg-Locke solution; c, effect of 10⁻⁶ g/ml PGE₂ in Ca-free Mg-Locke solution; d, recovery of membrane activity after perfusion with Ca-free Mg-Locke solution; e, effect of 10⁻⁶ g/ml PGE₂ on the membrane potential and outward current pulses in Ca-free Mg-Locke solution. Initial part of the record in e is control in Krebs solution.

DISCUSSION

PG action on the pregnant mouse myometrium. Low concentrations of PGE₂ increased the number of spikes and the frequency of the spikes in a train discharge and also generated spikes between the train discharges. When the concentration was increased, the depolarization of the membrane induced continuous spike discharges. A further increase in concentration of PGE₂ produced depolarization block of the generation of spikes. These changes in the membrane activity of the myometrium could also be observed on treatment with oxytocin (KURIYAMA, 1964; MARSHALL, 1968; OSA and TAGA, 1973). The action of PGE₂ also resembled that of oxytocin in that progesterone-dominated and early pregnant myometria of rat, mouse, rabbit, and human were less sensitive to oxytocin than the oestrogen-dominanted, late-stage of gestation and postpartum myometria (CALDEYRO-BARCIA and ALVAREZ, 1952; CALDEYRO-BARCIA et al., 1957; CSAPO, 1962). However, differences between PGE₂ and oxytocin on the membrane activity of mouse myometrium were demonstrated by OSA et al. (1974). For instance, prolonged
application of PGE₂ produced desensitization, but oxytocin did not. In the present experiments, PGE₂ was shown to have weak excitatory actions on the circular muscle compared with those on the longitudinal muscle, while oxytocin did not show any difference between longitudinal and circular muscles.

It is well-known that the hormonal state of the myometrium modifies not only its sensitivity to drugs, but also the membrane properties. The early pregnant myometrium shows irregular spike amplitudes and irregular appearance of the train discharges compared with those in the late pregnant or postpartum myometrium; the maximum rate of rise of the spike is greater in the early pregnant and midpregnant myometrium than in the late pregnant myometrium (Csapo and Kuriyama, 1963). It has been postulated that the possible mechanism underlying these phenomena is that the amount of fixed Ca in the membrane is controlled by progesterone and the action of oestrogen is thought to mobilize Ca ion from the membrane (see reviews of Marshall, 1962; Kuriyama, 1964; Abe, 1970).

Membrane property and PGE₂ actions on the pregnant mouse myometrium. Anomalous rectification of the membrane was seen on treatment with PGE₂ with excess K or on electrical displacement of the membrane potential, i.e., the amplitude of electrotonic potential evoked by inward current pulses was larger in the depolarized membrane than in the control. This might be caused by reduction of K-conductance. This is an interesting property of the membrane of the mouse myometrium since a reduction of K-conductance enlarges the amplitude of the spike and reduces the maximum rate of fall of the spike, thus producing larger contraction as observed on treatment with tetraethylammonium (OsA, 1974). However, reduction of the K-conductance might appear as a consequence of membrane depolarization, because when the membrane resistance was measured at the membrane potential level shifted to the level before the application of the drug during the second grade response, it was slightly reduced compared with the control, although during the third grade response, it was reduced.

It has already been postulated by many investigators that in myometrium, PG labilizes the bound Ca sequestered in the membranes and also accelerates the excitation-contraction coupling (Coceani and Wolfe, 1966; Clegg et al., 1966; Paton and Daniel, 1967). Recently, Carsten (1973a,b,c) reported that sarcoplasmic reticulum extracted from bovine myometrium released Ca on treatment with PGE₂. However, the concentration of PGE₂ used in her experiments was higher than in the present experiments.

The depolarization of membrane induced by PGE₂ was markedly suppressed in the Na-deficient Locke solution and also in Ca-free Locke solution. OsA et al. (1974) argued that depolarization of the membrane produced by PGE₂ depends on the ratio of [Ca]₀/[Na]₀. In cardiac muscle, Niedergerke and Orkand (1966) concluded that the ratio of [Ca]/[Na]₀ modifies the mechanical response by acting on the surface membrane. Presumably, a similar situation exists at the membrane in the mouse myometrium.
In conclusion, a tentative hypothesis is presented on the mode of action of PGE$_2$ on the mouse myometrium: A low concentration of PGE$_2$ mobilizes bound Ca at the membrane, thus depolarizing the membrane. As a consequence, the K-permeability is reduced and anomalous rectification of the membrane is seen. Increased concentration of PGE$_2$ increases the Na-permeability of the membrane, which is also governed by [Ca]$_{o}$. This might also increase the intracellular free Ca ions which activate the contractile protein, since PGE$_2$ produces a similar contracture on the K-induced contracture to that observed on treatment with carbachol.

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REFERENCES


