THE MECHANISM OF ACTION OF PROSTAGLANDIN $F_{2\alpha}$ ON THE SMOOTH MUSCLE OF GUINEA-PIG TAENIA COLI

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Abstract—The effect of prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) on isolated smooth muscle of guinea-pig taenia coli as related to cholinergic and adrenergic receptors of the muscle was investigated using the double sucrose-gap method. $\text{PGF}_{2\alpha}$ in the concentration of 1.0×10^{-6} g/ml caused and augmented the contraction of the smooth muscle by depolarizing the membrane, decreasing the membrane resistance, and increasing the frequency of spike generation. These actions were not abolished by atropine sulfate (10^{-7} g/ml), phentolamine (10^{-6} g/ml), propranolol (10^{-6} g/ml), respectively. $\text{PGF}_{2\alpha}$ had the same action as that of acetylcholine on the contraction of the smooth muscle, but the onset was slower and the duration longer. The inhibitory effects of adrenaline (10^{-7} g/ml), noradrenaline (10^{-7} g/ml), and isoproterenol (10^{-7} g/ml) were not suppressed by treatment with $\text{PGF}_{2\alpha}$. These results indicate that the effect of PG was not mediated either by the cholinergic nor the adrenergic receptors (α and β receptors), and it is suggested that $\text{PGF}_{2\alpha}$ may act on sites of the cell membrane other than adrenergic and cholinergic receptors to increase the membrane conductance as well as in the intracellular mechanism for the induction and augmentation of contraction of the taenia coli smooth muscle. The interrelationship of the PG and Ca ion is also discussed.

It is well known that prostaglandins (PGs) are widely distributed in animal tissues (1-7) and have many biological activities (8). The presence of PGs in the central and peripheral nervous systems of the human, ox and other mammals has been detected (3-5), but exact physiological roles are still unknown. The local presence and release of PGs in organs of smooth muscle by the stimulation of autonomic nerves have also been documented (9-11), but the mechanism of action of PGs on the contraction of smooth muscle is still obscure. Regarding the distribution of PGs in nervous system and release of same by autonomic nervous excitation with the mode of action of autonomic nerves controlling the intestinal smooth muscle mobility, it is reasonable to infer a interrelationship among chemical transmitters in the nerves, PGs and the muscular contraction. Bennet et al. (12) stated that PGE_1 and E_2 seemed to stimulate both the intrinsic cholinergic nerves and intestinal smooth muscle cells. Clegg et al. (13) elucidated the mechanism of action of Ketogenic PGs (E_1 and E_2) on the myometrium, by means of Kymographic, electrical field stimulation and single sucrose-gap methods, and concluded that PGE_1 and E_2 have two different kinds of effects; the direct and enhancement. The former is postulated to result from depolarization at the exposed cell membrane whereas the latter may result from facilitation of excitation contraction coupling. The present experiment was carried out by the application of a double sucrose-gap method to

investigate the mechanism of action of $\text{PGF}_{2\alpha}$ on the smooth muscle of taenia coli, particularly as related to cell membrane and cholinergic and adrenergic receptors.

MATERIALS AND METHODS

Guinea-pigs (non-pregnant) weighing 250 to 350 g were used. Under ethyl ether vapor anesthesia a strip of the guinea-pig taenia coli about 30–40 mm length was dissected from the caecum. The strip was suspended and bubbled with 100% O_2 in Tyrode solution (32°C) for about 40 min to an hour after which it was mounted in the double sucrose-gap chamber.

The double sucrose-gap method used was an essentially modified version of that described by Stämpfli (14), Julian et al (15) and Berger (16). The sucrose-gap chamber can be considered as three separated pools, with two end pools separated from the middle one by high resistance sucrose-gaps, and there is a rubber membrane between each neighbour pool and gap pools (Fig. 1). From left to right (Fig. 1), the pools are designated: current or I-pool; left sucrose-gap; center or C-pool; right sucrose gap; and voltage or V-pool, respectively. The muscle strip was mounted in the chamber through the channel (2.0 mm diameter) which runs across the central part of each pool, and exposed to Tyrode solution, isotonic sucrose, test solution, isotonic sucrose and to isotonic K_2SO_4 solution (see Fig. 1), respectively. One end of the muscle strip was tied and fixed to the supporting board, and the other end was tied to a fixed point of strain gauge in order to adjust the passive tension and to record the change of tension during the contraction of the muscle. The strip was stretched to a passive tension of 0.5 to 1.0 g.

FIG. 1. Schematic representation of the sucrose-gap chamber. Dotted areas represent sucrose-gap pools separated by rubber membranes. Rub. memb.; rubber membrane. (I): current pool. (C): center pool. (V): voltage pool. I: current. V: voltage. Lower part of the figure is electrical equivalent circuit of the chamber.

Solution for the three pools and the sucrose solution for gap pools flowed by gravity from the reservoir bottles into polyethylene tubes passed through a warming bath mounted closely to the chamber. The warming bath was circulated with 41°C water in such a way that the perfused solution in each pool would be maintained at $32 \pm 1^\circ\text{C}$ by a thermostat

FIG. 2. Experimental apparatus setup for the double sucrose-gap study. SGC: sucrose-gap chamber. T: Tyrode solution. S: isotonic sucrose solution. K: isotonic K_2SO_4 solution.

equipped circulator (Fig. 2). The rate of flow of the solution was 2.0 ml/min in the C-pool and 1.0 ml/min in the I and V-pools. The drugs and the PG were administered via a small vinyl tube with one end inserted in the C-pool, and the other end connected to a micro-infusion pump (Atom infusion pump AIP-2H), which controlled the administration of the drugs in a desired concentration and in a constant speed into the C-pool (see Fig. 2). The concentration given in the results is at of the final concentration of the drugs or PG in contact with the tissue. Three Ag-AgCl electrodes were connected to these three pools (I, C, V pools) by 3M KCL agar bridges. The tip of the electrode in each pool was located downstream in order to prevent the diffusion of KCL from contacting the tissue, particularly that in the center pool. The membrane potential was measured with a high input impedance preamplifier connected between the V-pool and ground (center pool). The resting membrane potential was established by exposing the V-pool end of the muscle strip to isotonic K_2SO_4 . Since the C-pool was maintained at virtual ground potential, the electrometer measured the potential between the inside of the muscle strip in the V-pool and the outside at the C-pool. Stimulation current pulses (3 sec duration) were applied to the tissue in the I-pool with alternating polarities at constant interval (10 sec frequency) when necessary. Details are given in Figs. 1 and 2.

A modified Tyrode solution of the following composition was used (mM): Na^+ , 137; K^+ , 2.7; Mg^{++} , 0.1; Ca^{++} , 1.8; Cl^- , 141.8; glucose, 5.5. The pH of the solution was kept

at 7.2–7.3 with tris-buffer. Isotonic sucrose solution was prepared by dissolving 92.5 g crystal sucrose (highly purified, Takeda, Japan) in 1000 ml of deionized, distilled water. The resulting sucrose solution had a conductivity of the order of $10^{-5} \text{ ohm}^{-1} \text{ cm}^{-1}$. Isotonic K_2SO_4 was in concentration of K_2SO_4 21.1 g per l (121.1 mM/l) of distilled water.

Drugs used in the present experiments were adrenaline-HCl (Sankyo Co. Japan), dl-noradrenaline-HCl (Sankyo Co. Japan), acetylcholine-Cl (Daiichi Co. Japan), atropine sulfate (Tanabe, Japan), and crystal prostaglandin $\text{F}_{2\alpha}$ (Ono Pharmaceut. Manuf. Co., Tokyo, Japan). As α and β blocking agents, phentolamine (Ciba), and l-propranolol (Sumitomo Chem. Co., Japan) were used. The drugs were dissolved and diluted to the required concentration in Tyrode solution and applied with syringes A, B and C to C-pool of the chamber (Fig. 2).

RESULTS

Responses of the tissue to $\text{PGF}_{2\alpha}$

When the tissue was mounted in the chamber and Tyrode solution was perfused in the three pools, spontaneous spikes (spike potential) were generally recorded. The

FIG. 3. Typical responses of the smooth muscle of guinea-pig taenia coli to $\text{PGF}_{2\alpha}$ (10^{-6} g/ml). A line in record: membrane potential changes. B line: membrane potential and electrotonic potential changes with application of stimulation current. Bottom line: current monitor. Bars indicate the application of $\text{PGF}_{2\alpha}$ (10^{-6} g/ml).

FIG. 4. Responses of the smooth muscle of guinea-pig taenia coli to $\text{PGF}_{2\alpha}$ (10^{-6} g/ml). Experimental conditions were the same as those cited in the Fig. 3. Here the depolarization effect of PG was not so distinct as that in Fig. 3.

resting potential of the membrane was 55 ± 5 mV. The spontaneous spikes were not influenced by depolarization. In this condition, $\text{PGF}_{2\alpha}$ enhanced the contraction response of the muscle while increasing the amplitude and the frequency of spike generation (Fig. 3A). The membrane was depolarized with decrease of membrane resistance, since the size of electrotonic potential decreased (Fig. 3B). Other preparations of taenia coli revealed little change in membrane potential when in contact with the PG, but the increase in spike frequency and the decrease in membrane resistance remained unaltered (Fig. 4). The taenia coli did not respond to the PG so fast as that to acetylcholine (ACh), and there was a latent period of 10–20 sec.

Actions of acetylcholine and cholinergic blockade on the effect of $\text{PGF}_{2\alpha}$

A typical effect of ACh on the smooth muscle of taenia coli can be seen in Fig. 5. ACh (10^{-6} g/ml) induced the muscle to contract by depolarization of membrane and burst of spikes, and the effect disappeared immediately when the ACh in the C-pool was washed out. Though the PG had the same contraction effect, the onset of the response was slower, but lasted longer than that of ACh.

FIG. 5. Comparison of effects of acetylcholine (ACh) and $\text{PGF}_{2\alpha}$. A line in record : changes of membrane potential under treatment with ACh (10^{-7} g/ml). B line : under treatment with $\text{PGF}_{2\alpha}$ (10^{-6} g/ml)—control. C line : under pretreatment with atropine sulfate (10^{-7} g/ml) for 10 min, the ACh effect was antagonized, but the PG effect was not abolished.

After perfusing atropine sulfate (10^{-7} g/ml) to C-pool for 10 min, the ACh (10^{-6} g/ml) effect was completely abolished, but there was no influence on the activating action of the PG on the muscle (Fig. 5C). The depolarization action of ACh was more distinct than that of the PG but shorter in the duration.

Actions of adrenaline, noradrenaline and adrenergic blockades on the effect of $\text{PGF}_{2\alpha}$

Adrenaline (10^{-7} g/ml) inhibited the spontaneous spike and hyperpolarized the membrane (Fig. 6A), but the inhibitory action of adrenaline was of short duration. Phen-tolamine (10^{-6} g/ml) and propranolol (10^{-6} g/ml) antagonized the α and β -effects of adrenaline, but did not inhibit the depolarizing and other activating effects of the PG (Fig. 6B and C). Both noradrenaline and isoproterenol had an inhibitory action on the smooth muscle, and these inhibitory actions could not be suppressed by PG. As shown in Fig. 7, the depolarizing activity of the PG was blocked transiently by noradrenaline and isoproterenol and the effect of PG was restored after the action of these two agents had worn off.

FIG. 6. Comparison of effects of adrenaline (Ad) and $\text{PGF}_{2\alpha}$. A line in record: changes of membrane potentials under treatment with Ad (10^{-7} g/ml) and $\text{PGF}_{2\alpha}$ (10^{-6} g/ml). B line: under pretreatment with phentolamine (10^{-6} g/ml) for 10 min. C line: under pretreatment with both phentolamine (10^{-6} g/ml) and propranolol (10^{-6} g/ml) for 10 min, the effects of Ad were abolished, but the effect of $\text{PGF}_{2\alpha}$ remained.

FIG. 7. Effects of $\text{PGF}_{2\alpha}$, isoproterenol and noradrenaline on the electrical activities of taenia coli smooth muscle. PG: $\text{PGF}_{2\alpha}$ (10^{-6} g/ml). I: isoproterenol (10^{-7} g/ml). NA: noradrenaline (10^{-7} g/ml). A line in record: PG control. B line: the PG effect was suppressed by I and recovered after the I effect wore off. C line: the PG effect was suppressed by NA and recovered after the NA effect had worn off.

DISCUSSION

The results of the experiment revealed that $\text{PGF}_{2\alpha}$ (10^{-6} g/ml) had the remarkable effect of inducing contraction of the smooth muscle of taenia coli. The effect was prominent in two respects. The first was manifested by the induction of the contraction of smooth muscle with depolarizing action on the membrane and an increase of the membrane conductance. The second was manifested by the induction and augmentation of contraction while increasing the frequency of spike generation. The depolarizing action of PG may not be required for the induction of contraction of the muscle, as the PG slightly depolarized the membrane of other preparations of taenia coli without any change of membrane conductance and spike frequency (Fig. 4). The depolarizing effect may be determined by the equilibrium potential of the PG. Bennett (17) estimated that the ACh equilibrium potential might be in the range of -20 to -26 mV. All the preparations of taenia coli used in the experiment had a resting membrane potential higher than

the equilibrium potential of ACh, so that ACh always showed a marked depolarizing effect. In the same way, when the resting membrane potential was near or even lower than the equilibrium potential of $\text{PGF}_{2\alpha}$ (45 to 50 mV), the depolarizing effect of the PG could not be observed and even reversal has to be considered. One evidence of this is seen in Fig. 4. In respect of the effects of depolarization, increase of membrane conductance, and increase of spike generation (18, 19), the actions of ACh and the PG were identical. But as the effect of PG was not influenced by atropine sulfate and the effects lasted longer, the actions of PG and ACh appeared to be quite different in nature. From this point of view, it can be concluded that there is no interaction of PG between cholinergic receptors and that the stimulating effect of $\text{PGF}_{2\alpha}$ on the intrinsic cholinergic nerves of taenia coli cannot be considered the same as that on intestinal smooth muscle as described by Bennet (17). The effect of catecholamines on the electrical and mechanical activities of the guinea-pig taenia coli were investigated extensively by Bülbiring and Tomita (20, 21). They stated that adrenaline hyperpolarized the membrane and blocked the spike discharge of the muscle, and concluded that the hyperpolarization is due to the increased K-conductance of the membrane (α -effect) and the blockade of spikes discharge is the result of the suppression of the pace maker potential (β -effect), and both effects were abolished by phentolamine (α -blockade) and propranolol (β -blockade), respectively. The present experiment gave the same results (Fig. 6), but the effect of PG was not abolished or inhibited by the adrenergic blockades, nor did PG (10^{-6} g/ml) suppress the inhibitory action of adrenaline (10^{-7} g/ml), noradrenaline (10^{-7} g/ml) and isoproterenol (10^{-7} g/ml) (Fig. 7). These results indicate that there is no interaction of PG between adrenergic receptors and the sites of adrenergic action. The results of experiment as shown in Fig. 7 may be explained by the fact that $\text{PGF}_{2\alpha}$ and catecholamines acted on different sites of the cell membrane and the result was the summation of actions of these two substances. When noradrenaline and isoproterenol were moderate in concentration (10^{-7} g/ml) the inhibitory action of these two amines was strong enough to overcome the contractor action of PG, so that the contraction caused by the PG was inhibited, and was restored after the noradrenaline and isoproterenol effects diminished, since the effect of PG was more long lasting. Carlson and Oro (22), and Strong and Bohr (23) concluded that PGE_1 lowers blood pressure by an action independent of catecholamine. Our present results confirm this conclusion.

The size of electrotonic potential of the cell of taenia coli smooth muscle was reduced as the cell was exposed to the PG. Kuriyama and Tomita (24) reported that a change in the size of electrotonic potential of smooth muscle cells of taenia coli indicates a change in the membrane resistance. By means of this analysis, the reduction of electrotonic potential of the smooth muscle cells caused by the PG expressed the decrease of membrane resistance of the cell caused by the PG, i.e. the increase of permeability of the membrane was induced by the action of PG. Changing the membrane permeability may be one of the most significant actions of PG. Thus, the direct effect described by Clegg et al (13) may not be due to the result of depolarization but rather to the increase of con-

ductance of the cell membrane. The fact that Ca ions play a role in controlling membrane conductance, and the spike in taenia coli is due to Ca ion entry (24), the interaction of $\text{PGF}_{2\alpha}$ and Ca ion may be reduced. Though there was no other evidence in the present experiments which would support this deduction, it is reasonable to assume that the effect of $\text{PGF}_{2\alpha}$ on the cell membrane is to decrease membrane resistance by mobilization of Ca ions at sites which normally fix Ca ions. This interrelationship of the PG and Ca may be a most interesting one for further investigation regarding the mechanism of the action of prostaglandins on the smooth muscle. There was a latent period (10–20 sec) in the onset of action of the PG and the PG action lasted longer in comparison with that of ACh. The intracellular mechanism of action of the PG cannot be disregarded.

It is concluded that the effect of PG is not mediated by the stimulation of cholinergic receptor nor the suppression of alpha and beta adrenergic receptors. $\text{PGF}_{2\alpha}$ may act on the special sites of the cell membrane to increase the membrane conductance or at specific receptors and on the intracellular mechanism for induction and augmentation of contraction of the taenia coli smooth muscle. The mobilization of Ca ions on the membrane and in the intracellular sarcoplasm by PG should also be considered.

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