MECHANISMS OF EFFECT OF PROTEIN KINASE C ACTIVATION ON ELECTRICAL AND CONTRACTILE ACTIVITY OF SMOOTH MUSCLE

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Definite progress has been made in recent years in the study of the intracellular communication pathway related to hydrolysis of membrane phosphoinositides and activation of protein kinase C (PKC). There is evidence that PKC activation by phorbol esters (PhE), which simulate the action of the secondary messenger diacylglycerol [5], essentially modifies the contractile responses of smooth muscles to the action of physiologically active substances (PAS) and of Ca^{++} ions [3, 15]. Effects of PKC activation differed in different types of muscles [7, 12]. Moreover, even in the same smooth muscle PhE could induce either contraction or relaxation, depending on the conditions [12]. However, no attempt was made in these studies to investigate the effect of PhE on electrical activity of smooth muscles (SM), which made the results more difficult to interpret. The first such investigation was undertaken by the present writers on SM of the taenia coli [2]. It was shown that PhE inhibits spontaneous electrical activity of SM of the taenia coli, inhibits the discharge of action potentials (AP) evoked by a depolarizing current, depresses the level of the initial mechanical contraction, and reduces the contractile responses to the action of PAS.

The aim of this investigation was to continue the study of these phenomena. Special attention was directed to the role of the K^+ channels and Na^+/H^+ exchange in the mechanisms of the inhibitory action of PhE on electrical and contractile activity of SM.

EXPERIMENTAL METHOD

The test objects were preparations of SM of the guinea pig taenia coli. The length of the muscle strip was 10-12 mm and its width 0.5-0.7 mm. To record mechanical and electrical activity simultaneously at rest and during stimulation, the double sucrose gap method, fully described in [1], was used.

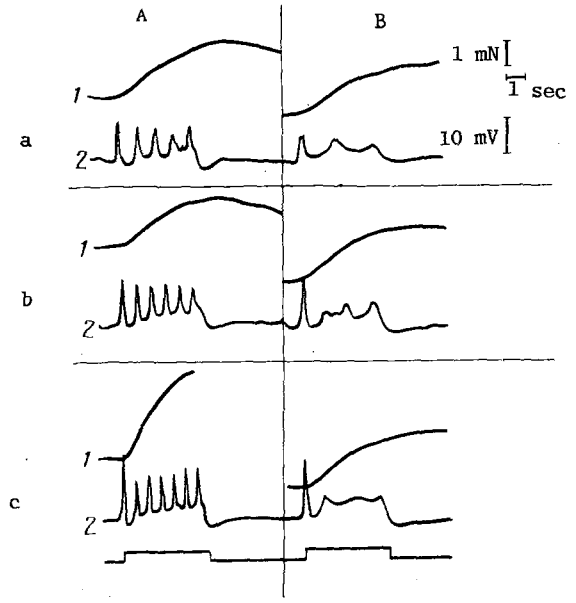

Fig. 1. Effect of TPA on changes in electrical and contractile activity of SM cells induced by depolarizing currents of different strength. A) AP evoked in control solution; a-c) strength of current 0.1, 0.2, and 0.35 μA, respectively. B) The same in the presence of TPA ($2 \cdot 10^{-8}$ M). Results of one of seven experiments shown. Here and in Figs. 2-4: 1) electrical activity; 2) contractile response. Marker of action of depolarizing current below.

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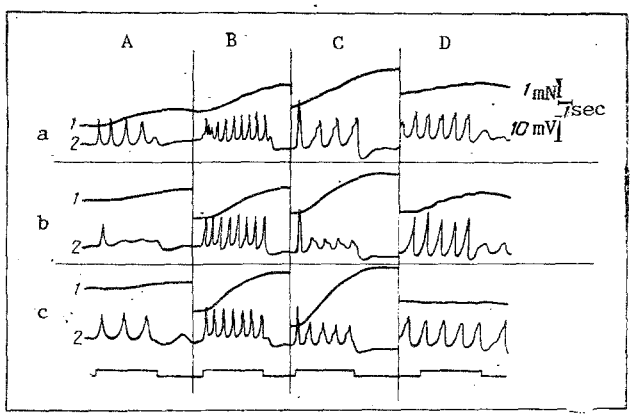

Fig. 2. Effect of TEA ($5 \cdot 10^{-3}$ M) and EIPA (10^{-5} M) on changes in electrical and contractile activity of SM cells induced by TPA. A-D) Results of four different experiments. a) AP and contractions in control solution, evoked by depolarizing current. A: b) In presence of TPA ($2 \cdot 10^{-8}$ M); c) on addition of TEA in the presence of TPA. B: b) In presence of TEA; c) on addition of TPA in presence of TEA. C: b) In presence of TPA ($2 \cdot 10^{-8}$ M); c) on addition of EIPA in presence of TPA. D: b) In presence of EIPA; c) on addition of TPA (10^{-7} M) in presence of EIPA. Remainder of legend as to Fig. 1. Results of two of 11 experiments with TEA and two of nine experiments with EIPA are shown.

Electrical signals were recorded by means of an FOR-2 camera from the screen of an S1-18 oscilloscope and on a KSP-4 automatic recording potentiometer. Contractile activity was recorded by means of a 6M × 1B mechanotron, under near-isometric conditions. The following solutions were used for external perfusion of the part of the strip to be studied.

1. Control Krebs' solution (in mM): NaCl, 133; KCl, 5.0; $MgCl_2$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1.4; glucose, 11.5; Tris-HCl, 15 (pH 7.35).

2. Control solution with addition of the test substances, namely: 12-o-tetradecanoyl-phorbol-13-acetate (TPA); 4β-phorbol-12-myristate-13-acetate (PMA); 4 -phorbol-12β-13-didecanoate (4α-PD); ethylisopropylamyloride (EIPA); tetraethylammonium chloride (TEA); monensin. PhE, EIPA, and monensin were dissolved in diethyl sulfoxide. The final concentration of the solvent in the experimental solution did not exceed 0.5% and affected neither the electrical nor the contractile activity of the muscle. The temperature of the solution was maintained at 36.5-37°C.

EXPERIMENTAL RESULTS

In concentrations of $2 \cdot 10^{-8}$ M and $5 \cdot 10^{-7}$ M, respectively, TPA and PMA inhibited the AP discharge arising in response to an above-threshold depolarizing current. In response to electrical stimulation, instead of a repeated response, only a single AP appeared. In most cases the amplitude of this AP showed little change compared with its initial level in normal Krebs' solution (Fig. 1B, b). Essentially PhE disturbed only the ability of the cells to generate a repeated response.

Unlike TPA and PMA, the inactive analog 4α-PD ($5 \cdot 10^{-7}$ M) did not affect SM activity (not shown). Consequently, the observed effects of TPA and TMA were in fact linked with PKC activation.

Depression of the electrical discharge by PhE may be due to elevation of the threshold of generation of the repeated response or to strengthening of the inactivation of Ca^{++} channels which arises after a single AP. In the first case the AP discharge ought to be restored if the strength of the depolarizing current is increased, whereas in the second case, a weaker stimulus ought to evoke a stronger response. In the present experiments neither an increase (Fig. 1B, c) nor a decrease (Fig. 1B, a) in the strength of the depolarizing current restored the repeated response.

One cause of inhibition of the electrical discharge by PhE may be an increase in potassium conductance of the membrane, or the membrane shunt formed by open K^+ channels prevents AP generation. This hypothesis was tested with the aid of the K^+-channel blocker TEA.

In a concentration of $5 \cdot 10^{-3}$ M, TEA on addition to a solution containing TPA ($2 \cdot 10^{-8}$ M) restored spontaneous and depolarization-evoked electrical activity (Fig. 2A). Since TEA stimulates spike activity of SM of the taenia coli in the control solution also, the order of the experiment was changed and PhE was added to the solution after the effect of TEA had become established. It will be clear from Fig. 2B that after treatment of SM with TEA (Fig. 2B, b), TPA had no significant effect on the repeated response evoked by the excitatory electrical stimulus (Fig. 2B, c). These data are evidence that inhibition of electrical ac-

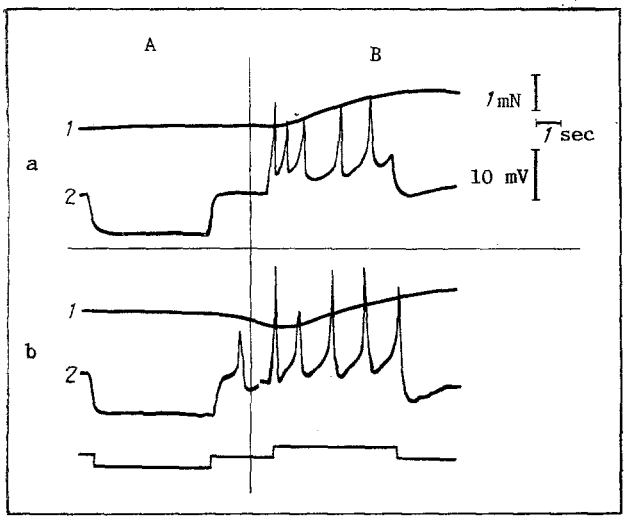

Fig. 3. Effect of EIPA on changes in electrical and contractile activity of SM cells evoked by depolarizing current, and passive shift of membrane potential induced by hyperpolarizing current. a) Passage shift of membrane potential and AP in control solution; b) in presence of EIPA (10^{-5}). Top left – marker of action of hyper-, top-right – and of depolarizing current. Remainder of legend as to Fig. 1. Results of one of eight experiments are shown.

tivity of the isolated SM strip in response to PhE is largely associated with an increase in potassium conductance of the membrane. To what can this increase of potassium conductance be attributed?

There is evidence in the literature that external application of PhE for intracellular injection of PKC can modify the Ca^{++}- [6] and K^+-currents [14] in neurons and certain other cells. Modification of the properties of the ionic channels may be the result of phosphorylation of membrane proteins controlling the ionic channels, or to covalent modification of the intermediate substrate in the regulatory chain, whose final link is a change in the state of the K^+-channels. Investigations have shown that activation of PKC in different cells stimulates Na^+/H^+-exchange [8]. This ion-transporting system in smooth-muscle cells is an important mechanism for the maintenance of the intracellular pH and the main way by which Na^+ enters the cells [13].

To study the possible role of Na^+/H^+-exchange in the effects of PhE, we used a PhE inhibitor, the amyloride derivative EIPA [10]. In a concentration of $5 \cdot 10^{-6}$ M EIPA increased the amplitude of AP during the first minutes of its action (Fig. 3B). In high concentrations ($2 \cdot 10^{-5}$ M), besides increasing the amplitude, EIPA also reduced the number of AP in the discharge arising in response to an electric current. EIPA had a stimulating action on spontaneous electrical activity (not illustrated) and on the break response after discontinuation of the steady hyperpolarizing effect (Fig. 3A,b). The amplitude of MP was unchanged by EIPA (not illustrated).

These effects of EIPA can be explained by reduction of the potassium conductance of the SM cell membrane. Unfortunately, there have been few studies of the effect of amyloride and its derivatives on ionic conductance of cell membranes. It has been shown that the outflow of K^+ is reduced by the action of EIPA on pancreatic cells [11].

Addition of EIPA to the perfusion solution after treatment of the SM preparation by PhE partially restored the AP discharge (Fig. 2C, c) and the "break" response to discontinuation of the hyperpolarizing current (not illustrated). A similar result was obtained when the order of application of the drugs was changed. As Fig. 2 shows, PhE in the presence of EIPA caused no significant changes in the repeated response or spontaneous electrical activity of the cells. In some experiments, however, a small decrease in amplitude of the AP was observed in response to the action of PhE preceded by EIPA.

It can be postulated on the basis of these results that activation of Na^+/H^+ exchange plays an essential role in the mechanism of action of PhE on SM.

During stimulation of Na^+/H^+ exchange, alkalification of the cytoplasm takes place and sodium accumulates in it [4]. Experiments on cardiomyocytes [9] showed that each of these factors can increase the potassium conductance of the membrane. We attempted to create a model of the situation by using NH_4Cl to make the cytoplasm alkaline, and the Na^+/H^+-ionophore monensin in order to increase the intracellular sodium concentration. Addition of 10 mM NH_4Cl to the control solution led to marked reduction of the amplitude of all AP in the discharge and to a significant fall of the anelectronic potentials. Monensin, in a concentration of 10^{-5} M caused inhibition of the repeated response without any significant

changes in amplitude of the single AP or of membrane resistance. Just as in experiments with PhE, this inhibition was not abolished by a change of strength of the depolarizing current.

It can thus be concluded from these results that the effects of PhE on electrical and contractile activity of SM cells of the taenia coli, associated with activation of PKC, are largely determined by an increase of potassium membrane conductance, probably due to activation of Na^+/H^+ exchange.

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