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ROLE OF PROTEIN KINASE C IN THE REGULATION OF ELECTRICAL AND CONTRACTILE ACTIVITY OF SMOOTH MUSCLE: EFFECT OF PHORBOL ESTER

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Much progress has been made in recent years in the study of the role of phosphorylation of membrane proteins, induced by protein kinase C (PKC), in the regulation of cellular functions [10]. Nevertheless, many important aspects of this problem still remain unexplained. In particular, the role of PKC in the regulation of smooth muscle functions remains unexplained. It has been shown [6-9, 11] that direct activation of PKC by phorbol esters, simulating the action of the secondary messenger diacylglycerol, led to considerable changes in the mechanical resting tension and, in some cases, to marked weakening, and in others to strengthening of the contractile responses of a muscle to the action of mediators or of external Ca^{++}. However, the authors cited confined their investigations to the contractile activity of muscles, and did not attempt to differentiate between the effect of activation of PKC on systems of electrically and chemically excitable calcium (Ca) channels.

In the investigation described below, conducted on strips of guinea pig taenia coli, changes in electrical and contractile activity of the smooth muscles under the influence of depolarizing and hyperpolarizing electrical stimuli, induced by phorbol ester, were recorded simultaneously for the first time. The effect of phorbol ester on the effect of neurotransmitters also was investigated under conditions when spike activity was inactivated by potassium depolarization of the membrane. This last factor enabled the role of PKC in the regulation of the receptor-controlled system of Ca channels to be detected.

EXPERIMENTAL METHOD

Preparations of the smooth muscle of the guinea pig taenia coli were used as the test objects. The length of the muscle strip was 10-12 mm and its width 0.5-0.7 mm. The double sucrose gap technique [2] was used to record mechanical and electrical activity simultaneously at rest and during stimulation. Electrical signals were recorded by FOR-2 camera from the screen of an S1-18 oscilloscope and also on the KSP-4 automatic writing potentiometer. Contractile activity was recorded by means of the 6MKh2B mechanotron under near-isometric

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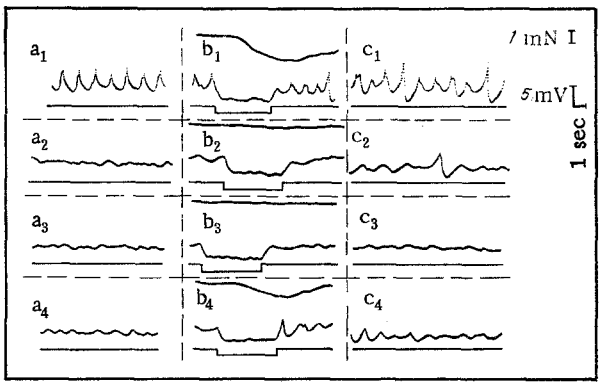

Fig. 1. Inhibitory effect of TPA ($2 \cdot 10^{-8}$ M) on spontaneous activity and BR of taenia coli smooth muscle. a_1-c_1) In control Krebs' solution; a_2-c_2) after 5 min of action of TPA; a_3-c_3) after 15 min of action of TPA; a_4-c_4) after rinsing preparation for 20 min with Krebs' solution. Top trace in middle column indicates mechanical tension of muscle in response to hyperpolarizing stimulus; middle trace indicates electrical activity; bottom trace is marker of changes in strength of polarizing current; downward deflection indicates time of application of hyperpolarizing current (0.2 μA). Here and in Fig. 2: top right — calibration signal and time marker (data for one of 5 experiments).

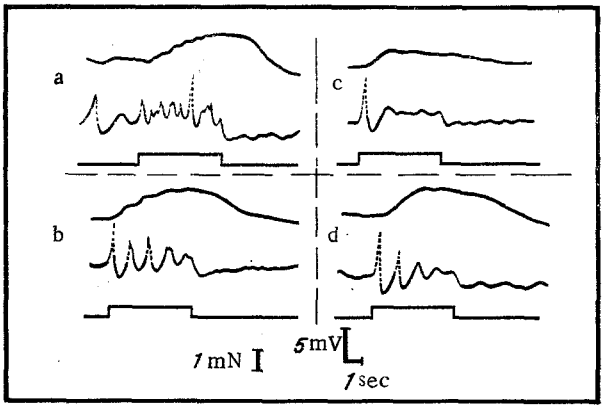

Fig. 2. Effect of TPA ($2 \cdot 10^{-8}$ M) on electrical and contractile activity of smooth muscle of taenia coli evoked by depolarizing current. a) In control Krebs' solution; b) at 5th minute of action of TPA; c) at 15th minute of action of TPA; c) at 20th minute of rinsing out preparation with Krebs' solution. Top curve — contractile activity; middle — electrical response; bottom — marker of polarizing current; upward deflection indicates time of application of depolarizing current (0.2 μA).

conditions. The following solutions were used for external perfusion of the test portion of the strip (in mM): 1) control Krebs' solution: NaCl 133, KCl 5.0, $MgCl_2$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.4, Tris-HCl 15, glucose 11.5, pH 7.35; 2) depolarizing solution: KCl 120, NaCl 47, $CaCl_2$ 0.4, $MgCl_2$ 1.2, Tris-HCl 15, glucose 11.5, pH 7.35; 3) control or "depolarizing" solutions with the addition of phorbol ester and (or) of the dry salts of one of the following physiologically active substances: acetylcholine hydrochloride, histamine dihydrochloride, and bradykinin triacetate.

The basic solution of phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) was made up in ethanol. The final concentration of ethanol in the experimental solution did not exceed 0.1%. In experiments on spontaneously active preparations the temperature in the control solution was maintained at 36.5-37°C. Experiments with the "depolarizing" solutions were carried out at room temperature (20-22°C).

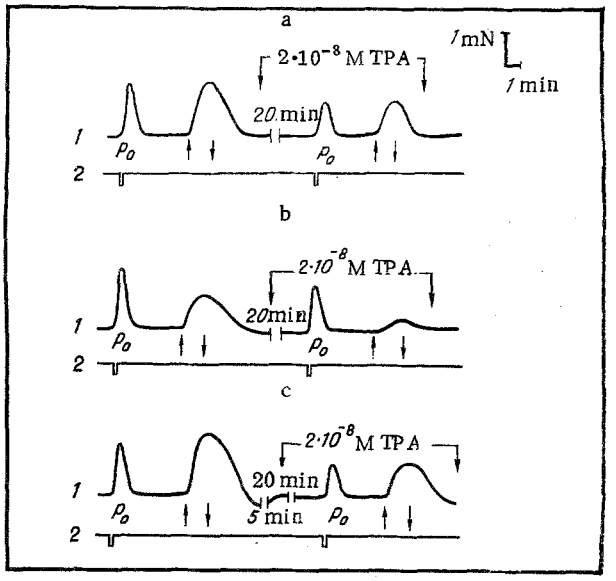

Fig. 3. Effect of TPA ($2 \cdot 10^{-8}$ M) on BR and contraction of depolarized strip of taenia coli, induced by physiologically active substances. a) Histamine (10^{-5} M), b) acetylcholine ($1.2 \cdot 10^{-5}$ M), c) bradykinin (10^{-5} M). 1) Trace showing changes in mechanical tension of muscle. Arrow pointing upward indicates beginning, downward — end of action of histamine, acetylcholine, and bradykinin. Arrows above trace indicate beginning and end of action of TPA; numbers under traces show time (in min). 2) Marker of polarizing current; downward deflection indicates time of application of hyperpolarizing current (2.6 μA). Top right — calibration signal and time marker (results of one of 14 experiments with acetylcholine, eight with histamine, and four with bradykinin).

EXPERIMENTAL RESULTS

In a concentration of $2 \cdot 10^{-8}$ M TPA caused a distinct fall of mechanical tension (tone) of the strip at rest (not illustrated) during the first few minutes of its action, like that observed previously [15] in experiments with exposure of smooth muscles of the guinea pig small intestine and trachea to the action of phorbol esters. It will be clear from Fig. 1 (a_1-a_3) that this relaxation of the "resting" muscle is based on inhibition of its spontaneous electrical activity.

Hyperpolarization of the membrane by a direct current in the control solution led to cessation of spontaneous electrical activity and lowering of the tone of the preparation (Fig. 1, b_1), which were temporarily intensified after disconnection of the current (Fig. 1, b_1-c_1) — the so-called "break" (post hyperpolarization) response (BR) appeared. There is reason to suppose [6] that it is based on reactivation of those voltage-dependent Ca channels which were in the inactivated state at the original membrane potential ("resting inactivation"). It will be clear from Fig. 1 that BR initially was reduced under the influence of treatment of the preparation with TPA (Fig. 1, b_2-c_2), but later (after 15 min) it completely disappeared (Fig. 1, b_3-c_3), although the magnitude of the hyperpolarization shift of potential induced by the direct current (Fig. 1, b_2-b_3) was virtually unchanged. The effect of TPA was long-lasting: after rinsing the preparation for 20 min spontaneous activity still remained depressed, whereas BR was only partially restored (Fig. 1, a_4-c_4). Inhibition of BR under the influence of TPA is evidence against the possible attribution of its effect to potentiation of "resting inactivation" of the Ca channels. In that case prolonged strong membrane hyperpolarization, which was used in the experiment (not illustrated), ought to have evoked a well defined BR, such as occurs, for example, on inactivation of Ca channels due to potassium depolarization of the membrane.

The effect of TPA on changes in electrical and mechanical activity of the smooth muscle induced by a depolarizing current is shown in Fig. 2. In the control solution (a) the depolarizing current induced an increase in the frequency of spontaneous electrical activity,

accompanied by a contractile response. This response was unconnected with activation of nerve endings in the muscle strip, because it was not abolished by 10^{-6} M tetrodotoxin (not illustrated). After the action of $2 \cdot 10^{-8}$ M TPA for 5 min (b) spontaneous activity ceased already, but the preparation was still able to respond to depolarization by a second discharge of action potentials and by normal contractions. After another 10 min (c) the same depolarizing stimulus evoked only a single action potential instead of a repeated response. However, the amplitude of this action potential was not reduced compared with the initial potential (b) and, consequently, only the ability of the preparation to generate a repeated response was disturbed. After rinsing of the preparation for 20 min the repeated response was restored although spontaneous activity was still absent.

The effect of TPA on the ability of smooth muscle to respond to acetylcholine, histamine, and bradykinin was studied during perfusion of the preparation with the control salt solution and during long-term potassium (120 mM KCl) membrane depolarization, which completely suppressed action potential generation in the smooth-muscle cells.

In the first case $2 \cdot 10^{-8}$ M TPA caused inhibition of both electrical and contractile responses of the strip (not illustrated) to bradykinin (10^{-7} M), acetylcholine ($1.2 \cdot 10^{-7}$ M), or histamine (10^{-7} M). However, these results were difficult to interpret because TPA, as we saw (Fig. 2), inhibits spike discharges arising in response to direct membrane depolarization by an electric current, not effected through receptors, also. We therefore set up experiments differently, with the Ca channels responsible for action potential generation in a state of inactivation. Under these conditions contractile responses to the transmitters were due to inflow of Ca^{++} into the cells only via receptor-controlled Ca channels, effectively blocked by D-600 [3, 4] or nicardipine (present investigation; not illustrated).

It will be clear from Fig. 3 that treatment of the depolarized smooth muscle with $2 \cdot 10^{-8}$ M TPA reduced its contractile responses to histamine ($1 \cdot 10^{-5}$ M), acetylcholine ($1.2 \cdot 10^{-5}$ M), and bradykinin (10^{-5} M). The degree of this inhibition varied in different experiments, but a distinct fall of amplitude of the responses was observed in all cases without exception.

Besides inhibiting the effect of physiologically active substances, TPA also reduced the magnitude of BR arising after blocking of the hyperpolarizing direct current (a) by PKC. The nature of these BR was studied previously [4-6]: they are based on reactivation of voltage-dependent Ca channels converted into a slow inactivation state under the influence of long-term potassium membrane depolarization. Suppression of BR in the depolarized muscle by TPA is evidently similar in nature to the inhibition of BR in the control solution (Fig. 1): in both cases activation of PKC by phorbol ester has an inhibitory effect on voltage-dependent ionic channels responsible for spike discharge generation and for contraction.

The results are thus evidence that PKC can exert a modulating effect on both systems of ion transport through the membrane of smooth muscle cells: the system of voltage-dependent channels responsible for spike activity of the muscle and the system of receptor-controlled Ca channels, transmitting the signal from membrane receptors inside the cell.

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