EFFECT OF CALMIDOZOLIUM (R 24571) ON THE ELECTRICAL AND CONTRACTILE PROPERTIES OF SMOOTH MUSCLE CELLS OF THE GUINEA PIG URETER

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Contractions in smooth muscle cells (SMC) are largely triggered and maintained by Ca^{++} ions, entering through voltage-dependent Ca-channels of the plasma membrane. A central role in the system of excitation–contraction coupling in SMC is played by Ca-binding proteins and, in particular, by calmodulin (CM) [5].

The writers showed previously [2] that the phenothiazine derivatives trifluperazine and chlorpromazine, known to be inhibitors of CM-dependent processes [8], depressed the electrical and contractile activity of SMC of the guinea pig ureter.

The recently synthesized preparation calmidozolium (CMD) is the most active of all known antagonists of CM [4, 7]. There is no information in the literature on the effect of this drug on electromechanical coupling in SMC.

In this paper we give the results of a study of the effect of CMD on electrogenesis and contraction of SMC.

EXPERIMENTAL METHOD

Experiments were carried out on isolated segments of the guinea pig ureter 10-12 mm long. The double sucrose gap method [1] was used for simultaneous stimulation and recording of the membrane potential and contractile activity.

Isolated muscle strips were immersed in Krebs' solution (KS) of the following composition (in mM): NaCl – 120.4, KCl – 6.9, $NaHCO_3$ – 15.5, $MgCl_2$ – 1.2, $Na_2H_2PO_4$ – 1.2, $CaCl_2$ – 2.5, glucose – 11.5, at pH 7.35, and at a constant temperature (36.5-37°C). The testing solutions were made up in KS with the addition of CMD (R 24571, from Boehringer, West Germany) in concentrations of 0.1 to 5 μM, or of the calcium ionophore A 23187 (Sigma, USA) in concentrations of 0.1 to 1 μM. The initial solutions of CMD and the ionophore were obtained by dissolving the dry substance in methanol. The alcohol concentration in the test solutions did not exceed 0.1%, which did not affect electrical activity or contraction of SMC of the ureter.

EXPERIMENTAL RESULTS

After perfusion of the preparation with normal KS for 60 min the typical electrical and contractile activity of these cells was recorded (Fig. 1). CMD, in micromolar concentrations, inhibited the spike component and the wave on the action potential (AP) plateau and reduced the force of contraction of SMC without any significant effect on the AP plateau. The effect of the inhibitor depended on its concentration. Total suppression of the fast components of AP and contractions took place with a concentration of 5 μM.

Concentrations causing inhibition by 50%, and determined from dose–effect curves were 1 μM for contraction and the number of oscillations on the AP plateau, and 3 μM for the amplitude of the fast components.

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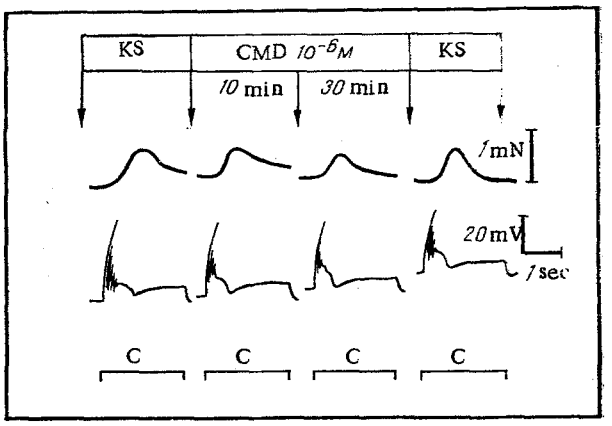

Fig. 1. Effect of CMD on electrical and contractile properties of SMC of the isolated guinea pig ureter. From top to bottom: contractile response, catelectrotonic AP, trace of depolarizing current (C). Arrows indicate beginning and end of the action of KS and CMD (10th and 30th minutes). Time marker and calibration signal on right.

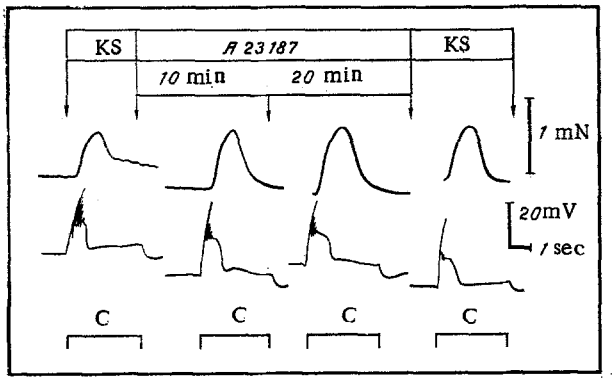

Fig. 2. Effect of calcium ionophore A 23187 ($8 \cdot 10^{-7}$ M) on electrical and contractile parameters of SMC of guinea pig ureter. Arrows indicate beginning and end of action of KS and A 23187 (10th and 20th minutes). Remainder of legend as to Fig. 1.

The resting potential and membrane resistance, estimated from the amplitude of the anelectrotonic potential were unchanged during exposure to CMD for 30 min.

To study the mechanisms lying at the basis of inhibition of ureteric contractions, the calcium ionophore A 23187 was used. Its addition to normal KS (0.8 μM) led to a significant increase in the force of contraction (by 62.0 ± 9.8%) and to a decrease in amplitude of the oscillations on the AP plateau of SMC (by 27.0 ± 9.2%; Fig. 2).

An increase in the contractile response, although by a lesser degree, also was observed when the ionophore was applied after previous treatment with the CM inhibitor (Fig. 3). CMD and the ionophore, when acting together, inhibited the fast components of AP by a greater degree than each of them separately.

Perfusion of the preparation with a solution containing the ionophore A 23187 (0.8 μM) and the ionophore + CMD (1 μM) did not change the resting potential or membrane resistance.

CMD inhibited the fast components of AP but had no significant effect on the AP plateau of SMC. The spike component and the oscillations on the AP plateau of the ureter are known to be produced by activation of fast voltage-dependent Ca-channels of SMC. The results indicate that one component of the Ca-channel of the SMC membrane, with a structural or regulatory function, is a protein containing hydrophobic groups, similar to those exposed in the CM molecule on interaction with Ca^{++}. Binding of CMD with these groups leads to a disturbance of conductance of the Ca channel.

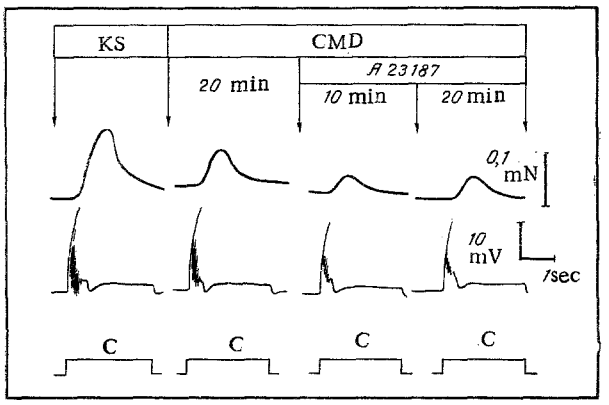

Fig. 3. Effect of addition of ionophore A 23187 ($8 \cdot 10^{-7}$ M) on inhibitory effect of CMD (10^{-6} M). Arrows indicate beginning and end of action of KS, CMD (10th minute), and of A 23187 together with CMD (10th and 20th minutes). Remainder of legend as to Fig. 1.

The conclusion is in agreement with recent studies [6] showing that the CM antagonist inhibits binding of ^{3}H-nitrendipine with Ca-channels.

These observations suggest that CM or a CM-like protein is present in the composition of the Ca-channel of the plasma membrane of SMC.

Inhibition of contraction by the action of CMD may be due both to a decrease in the quantity of Ca entering the cell during the AP and inhibition of CM-dependent protein kinase of the myosin light chains. The possibility of this last effect has been demonstrated in experiments in vitro on taenia coli preparations exposed to the action of chlorpromazine [3].

The fact that the Ca ionophore in the presence of CMD increased the amplitude of contraction points to a predominant effect of a reduction of the Ca currents during the action of the inhibitor.

The increase in contraction in the presence of the ionophore may be connected with an increase in the Ca^{++} concentration in the intracellular depots, which are mobilized in response to excitation of SMC.

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