ELECTROMECHANICAL COUPLING IN SMOOTH-MUSCLE CELLS OF THE URETER STUDIED WITH THE AID OF PHENOTHIAZINES

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Secretion of hormones and mediators and activation of thrombogenesis and smooth-muscle contraction are by no means a complete list of Ca-dependent processes controlled by the active complex of calcium with calmodulin (CaM) [4].

There is evidence of the leading role of CaM in triggering of smooth muscle contraction [3-10], but the role of CaM in electromechanical coupling processes in smooth-muscle cells (SMC) has not been investigated. Derivatives of the phenothiazine series, namely trifluoperazine (TFP) and chlorpromazine (CP), which "block" the biological effect of CaM [3, 8, 9], have been extensively used in the study of CaM-dependent processes, including phosphorylation of the myosin light chain — the key mechanism of SMC contraction.

In the investigation described below, these drugs were used to analyze the electrical and contractile properties of SMC.

EXPERIMENTAL METHOD

Experiments were carried out on isolated guinea pig ureters. The double sucrose gap method was used for simultaneous stimulation and recording of changes in membrane potential and mechanical contraction [1].

The following solutions were used. 1) Normal Krebs' solution (in mM): NaCl 120.4, KCl 6.9, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.5; 2) CaM inhibitors in Krebs' solution: CP in concentrations of 10^{-7}-10^{-4} M (USSR origin), TFP in concentrations of $5 \cdot 10^{-8}$-$2 \cdot 10^{-5}$ M (from Smith, Kline and French, India); 3) Krebs' solutions with Ca^{++} in concentrations of 10^{-4}-10^{-2} M.

EXPERIMENTAL RESULTS

CP and TFP in concentrations above 10^{-6} M inhibit both electrical and mechanical activity of SMC or even cause their complete disappearance. A special feature of the action of the phenothiazines is exhibited in the form of dissociated changes in the electrical complex and mechanical response (Fig. 1). The contractile response to depolarizing current was depressed earlier and in the presence of lower concentrations of inhibitors.

The action of phenothiazines, incidentally, developed slowly and inhibition did not reach a maximum until the 10th minute. When the testing solution was replaced by normal Krebs' solution, the blocking effect did not disappear at once. Moreover, during the first 5 min of rinsing of the preparations, with concentrations of inhibitors of under EC 100, inhibition of spike activity and of contraction was potentiated.

To clarify the mechanism of depression of electrical and mechanical activity, antagonistic relationships of phenothiazines and Ca^{++} with CaM were used.

It will be clear from Fig. 2 that TFP and CP, in a dose inhibiting by half spike activity and contraction, shift the dose—effect curve for Ca^{++} toward higher concentrations of the

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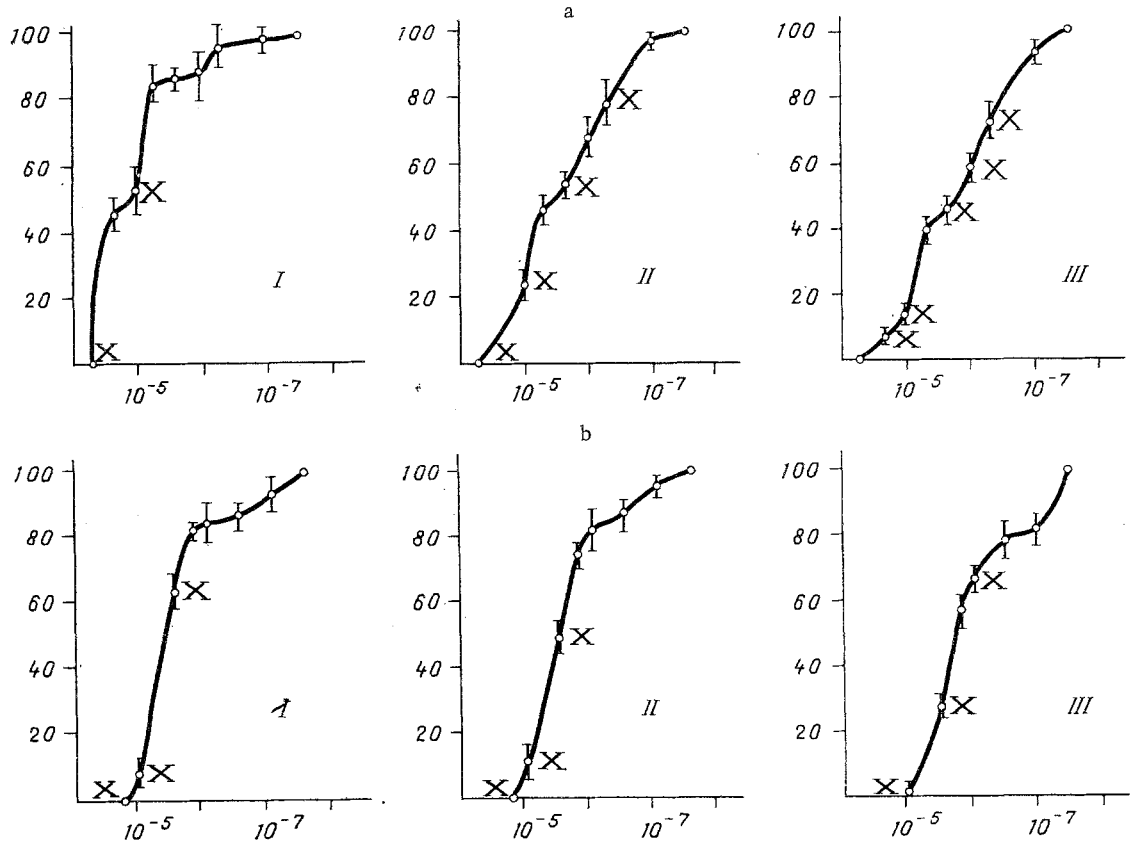

Fig. 1. Effect of phenothiazines on electrical and mechanical activity of SMC of ureter. a) CP; b) TFP. I) Amplitude of action potentials; II) number of action potentials; III) amplitude of contractions. Abscissa, dose of phenothiazine (in M); ordinate, effect (in % of maximal). Asterisk indicates significant differences between points on curve ($P < 0.05$, $n = 9$).

latter. On titration of Ca^{++} in the presence of inhibitors ($2 \cdot 10^{-6}$ M) electrical and mechanical activity of SMC was restored to half its initial value in the presence of Ca^{++} in concentrations of $3 \cdot 10^{-3}$ M with TFP and of $6 \cdot 10^{-3}$ M with CP. Analysis of the time course of inhibition and reactivation processes shows that definite differences exist in the mechanism of the effect of these drugs. When inhibitors were used in a dose causing 50% inhibition of electrical activity, the Ca^{++} concentrations needed to reactivate contractile and spike activity by half were the same for CP but not for TFP. In the case with TFP, electrical activity required a lower concentration of Ca^{++} for its restoration than contractile activity.

These results can tentatively be explained on the grounds that CP exerts its action by a competitive mechanism. The slow development of the effect, preceding inhibition of contraction without any visible change in spike activity, and potentiation of the inhibitory effect at the beginning of rinsing indicate the probable location of the CP "target" inside the cells. TFP evidently interacts noncompetitively with CaM, as shown by the shift of the dose–effect curves for Ca^{++} downward and to the right.

The results show that CaM mediates activation of the contractile apparatus in SMC of the ureter.

Both inhibitors used depress the rapid components of the electrical complex in SMC. This may be the result of their effect on conduction processes in the Ca channels of the membrane: either their blocking or disturbance of processes of modification of the channels connected with their activation. The latter hypothesis is supported by the kinetics of inhibition and reactivation, which differs from their kinetics during the action of blockers

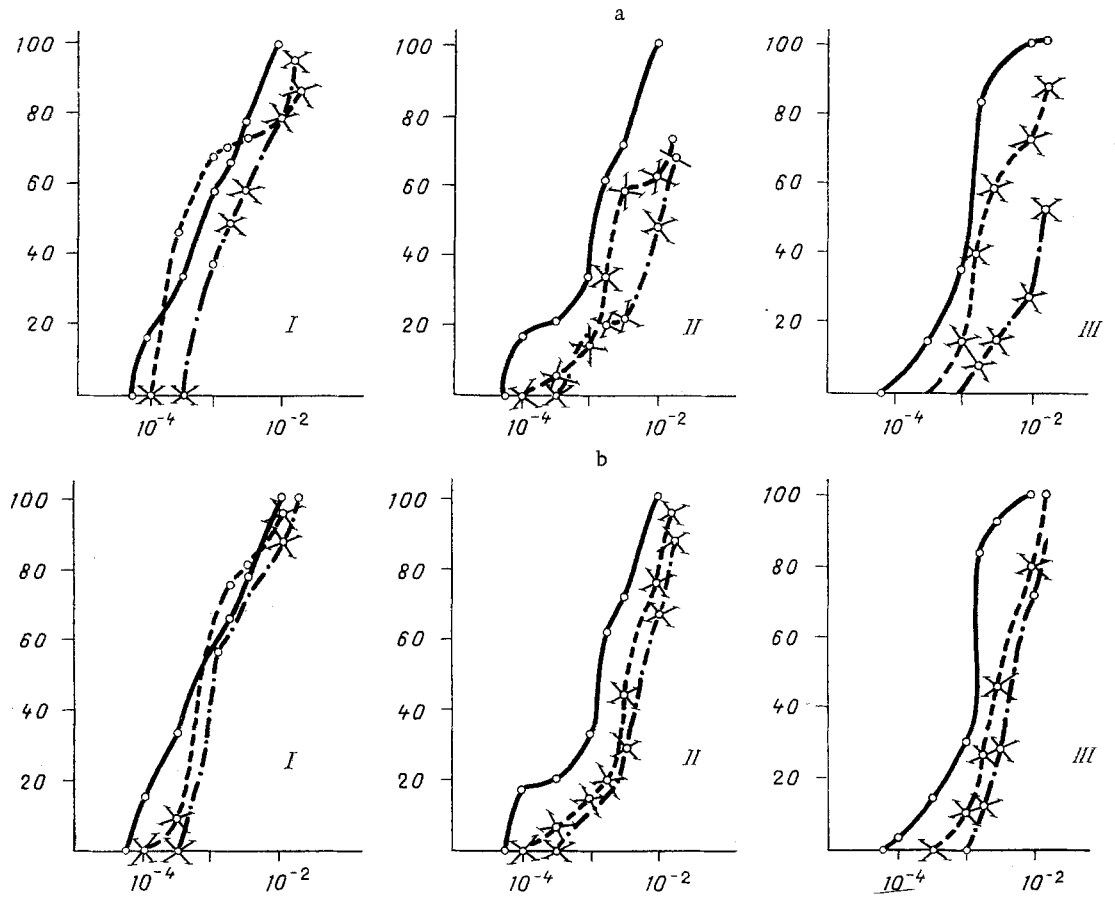

Fig. 2. Effect of phenothiazines and Ca^{++} on electrical and mechanical activity of SMC of ureter. a) CP; b) TFP. Continuous line shows effect of Ca^{++}; broken line — effect of phenothiazines in concentration of $2 \cdot 10^{-6}$ M; dot-and-dash line — the same, in concentration of $6 \cdot 10^{-6}$ M. Abscissa, Ca^{++} concentration (in M); ordinate, effect (in % of maximal). Asterisk indicates significant differences between points on curves 2 and 1, 3 and 2 ($P < 0.05$, $n = 9$). Remainder of legend as in Fig. 1.

of Ca channels [2]. The possibility cannot be ruled out that the active state of the channels is regulated by the Ca—CaM complex.

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