EFFECT OF PAPAVERINE ON TONE AND CONTRACTIONS OF THE DEPOLARIZED SMOOTH MUSCLE OF THE GUINEA PIG taenia coli

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Experiments were carried out on isolated strips of guinea pig taenia coli. The smooth muscle was depolarized in a solution with high potassium concentration (120 mM KCl). The effect of papaverine (in concentrations of 10^{-5} to 3.10^{-5} g/ml) on the tone and off-response to a prolonged and strong hyperpolarizing current was investigated on the denervated muscle. Papaverine was found: 1) to abolish contractile responses to application of histamine, bradykinin, and acetylcholine; 2) to reduce the tone of the depolarized muscle and abolish the effect of an increase in the Ca^{++} concentration in the external medium on muscle tone; 3) to have no effect on the amplitude and velocity of the ascending phase of the off-response; 4) to accelerate the descending phase of the off-response. The following hypotheses are put forward to explain the result: 1) in the cell membrane there are chemically excitable calcium channels which are blocked by papaverine; 2) in the membrane there are calcium leakage channels responsible for the maintenance of tone and blocked by papaverine; 3) papaverine has negligible effect on electrically excitable calcium channels.

KEY WORDS: depolarized smooth muscle; tone; contractile off-response; calcium channels.

It was postulated previously [3] that besides electrically excitable calcium channels responsible for action potential generation [8], chemically excitable calcium channels also exist in the surface membrane of smooth muscles, and these are activated by the action of biologically active substances (BAS) —acetylcholine, histamine, and bradykinin — on the corresponding receptors. Activation of chemically excitable calcium channels in a "pure" form can be observed as a result of the action of BAS on depolarized muscle [1, 4-6, 9], for under these conditions the electrically excitable channels are inactivated. The inactivation of these channels can be abolished by passing a hyperpolarizing current through the membrane; the contractile on-response (OR) to the current is evidently due to the inward flow of Ca^{++} through these channels. Both the contractile effects of BAS and the OR are abolished by compound D-600 and manganese ions [3, 4]. Local anesthetics (procaine, trimecaine, QX-572), inhibit the effects of BAS much more strongly than OR [3].

The present investigation shows that even more effective separation of chemically and electrically excitable calcium channels can be obtained with the aid of papaverine. Data important for the understanding of the spasmolytic action of papaverine on smooth muscle also are described.

EXPERIMENTAL METHOD

Experiments were carried out on isolated strips of the guinea pig taenia coli (1.5-2 cm long, 300-500 μ wide). For simultaneous stimulation and recording changes in membrane potential, the double sucrose bridge method was used. The construction of the experimental chamber was described previously [2]. Measurements of mechanical activity of the strips were made under isometric conditions by means of the 6MKh-IS mechanotron.

The following solutions were used: (in mM): a) normal Krebs' solution: NaCl 120.7, KCl 5.9, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5; b) potassium (depolarizing) solution: KCl 120, NaCl 47.7, $NaHCO_3$ 3.6, $CaCl_2$ 0.4, glucose 11.5. The pH of the solutions was 7.3. All experiments were carried out at a temperature of 22°C. These conditions were described previously [1].

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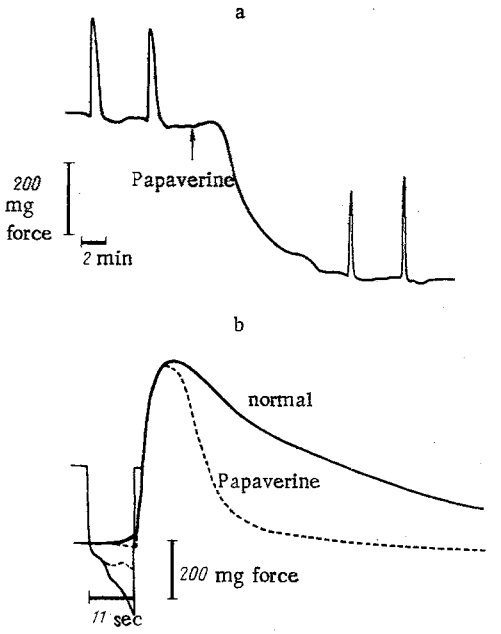

Fig. 1. Effect of papaverine on tone and OR of depolarized smooth muscle. a) Change in tone and OR under the influence of papaverine marked by arrow. b) Comparison of two OR (before and after action of papaverine). Changes of potential in artificial node during application of hyperpolarizing current shown on left (thin line).

EXPERIMENTAL RESULTS

The experiments were carried out as follows. The muscle was kept initially in normal Krebs' solution. Replacement of the Krebs' solution by the depolarizing solution caused potassium contracture of the muscle (not shown in the illustrations). After the end of the physical part of the potassium contracture, the muscle tone reverted to its previous resting level [6]. Against this background 11-sec pulses of hyperpolarizing current were applied and the OR was recorded (Fig. 1a). The addition of papaverine to the potassium solution in concentrations of 10^{-5} to 3.10^{-5} g/ml caused the muscle tone to decrease (Fig. 1a). In 2 of the 17 experiments the decrease in tone was preceded by a very small increase. After establishment of the new level of tone in papaverine, 11-sec pulses of hyperpolarizing current of the same strength were again applied (Fig. 1a). The ascending phase of the muscle OR in papaverine solution was virtually indistinguishable from the ascending phase of OR of the same muscle in potassium solution. It relaxed much faster. OR of a muscle in normal potassium solution and in the presence of papaverine are shown in Fig. 1b superposed on each other. The amplitude of OR remained unchanged in 14 of the 17 experiments and in the other three it was slightly reduced. In higher concentrations of papaverine (5.10^{-5} g/ml or higher) the amplitude of OR was always reduced.

A typical response of the muscle to addition of histamine (2.10^{-6} g/ml) in normal potassium solution is shown in Fig. 2a. In Fig. 2b OR of the muscle in normal potassium solution and in solution with the addition of histamine can be compared. Some lengthening of the phase of relaxation of OR through the action of histamine can be seen. The addition of papaverine to the potassium solution completely abolished the effects of histamine (Fig. 2c); the OR against the background of the combined action of papaverine and histamine, moreover, were virtually indistinguishable from OR in papaverine solution (Fig. 2c, d). In other words, papaverine abolished not only the contractile effect of histamine, but also its effect on OR. Similar results were obtained with acetylcholine (10^{-5} g/ml) and bradykinin (10^{-5} g/ml).

The blocking by papaverine of contractions evoked by BAS and the preservation of OR are evidence in support of the earlier view that there are two groups of calcium channels in the membrane of smooth-muscle cells.

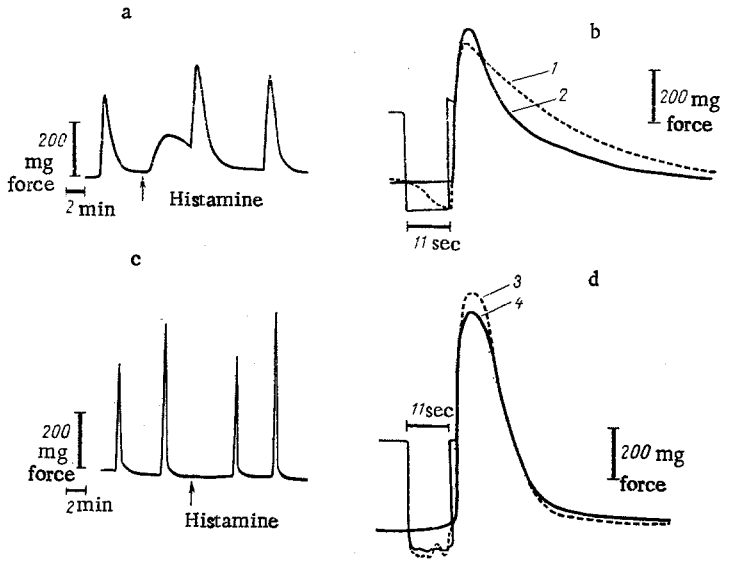

Fig. 2. Effect of papaverine and histamine on tone and OR of muscle. a) Response of muscle to application of histamine (2.10^{-6} g/ml); b) changes in OR against the background of action of histamine. Thin lines on left show changes in potential in artificial node; c) absence of contractile response of muscle to application of histamine after preliminary action of papaverine (10^{-5} g/ml); d) comparison of 2 OR (after preliminary action of papaverine and of papaverine with histamine). 1) Histamine 2) normal 3) papaverine + histamine, 4) papaverine.

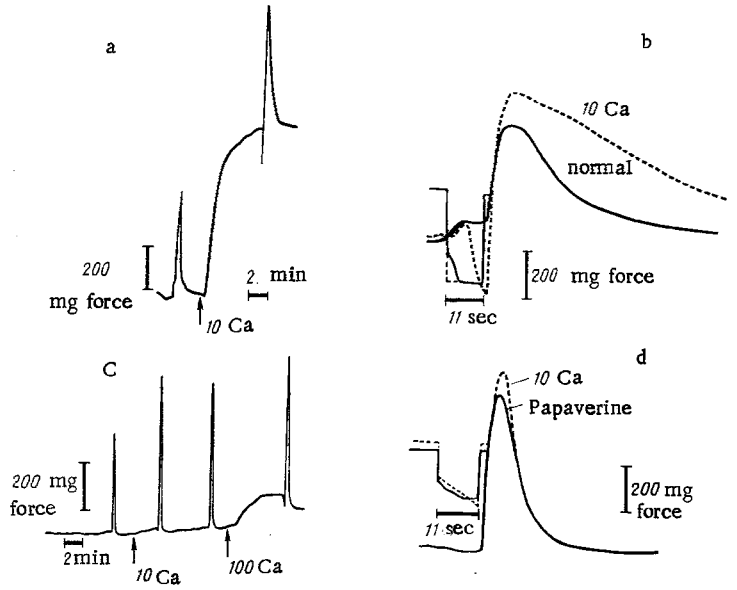

Fig. 3. Effect of increase in external Ca^{++} concentration on tone of muscle and its OR under norm conditions and after preliminary action of papaverine. a) Change in muscle tone and OR following tenfold increase in Ca^{++} concentration; b) comparison of 2 OR in potassium solution with normal and increased Ca^{++} concentration; c) 10- and 100-fold increase in external Ca^{++} concentration after preliminary action of papaverine. Record obtained 20 min after application of papaverine; d) comparison of 2 OR (after preliminary action of papaverine and papaverine with a tenfold increase in Ca^{++}).

Papaverine evidently selectively blocks the chemically excitable calcium channels which are activated by BAS but has virtually no effect on channels activated by an electric field. These findings are in agreement with the results of Imai and Takeda [7], who showed that papaverine does not block spike activity in the taenia coli.

Acceleration of the phase of relaxation of OR after the preliminary action of papaverine can be explained by several mechanisms: both by acceleration of the outflow of Ca^{++} into the external medium, and by the acceleration of its binding by intracellular depots. The results obtained do not provide a direct answer to this question.

To shed light on the mechanism of the decrease in tone under the influence of papaverine, experiments were carried out in which the external Ca^{++} concentration was changed. Removal of Ca^{++} from the normal potassium solution led to a sharp decrease in tone and complete disappearance of the OR. A tenfold increase in the Ca^{++} concentration caused a marked increase in muscle tone, which was maintained at a high level throughout the period of application of the solution with increased Ca^{++} concentration (Fig. 3a). OR increased in amplitude compared with normally (Fig. 3a, b); the phase of relaxation was lengthened under these circumstances just as after preliminary application of histamine (compare with Fig. 2b). A tenfold increase in the external Ca^{++} concentration after preliminary action of papaverine (4 experiments) did not cause an increase in muscle tone (Fig. 3c) and did not delay the phase of relaxation of OR, although their amplitude increased (Fig. 3c, d). Even a 100-fold increase in the Ca^{++} concentration in the solution led only to a very small increase in muscle tone (Fig. 3c).

The results are evidence that papaverine blocks the flow of Ca^{++} into the cell responsible for the maintenance of muscle tone. Evidently in the depolarized membrane there is a steady Ca^{++} flow through permanently open calcium channels. The blocking of these channels by papaverine may perhaps be the cause of the decrease in muscle tone. In this case an increase in the extracellular Ca^{++} concentration would not lead to any increase in tone.

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