EFFECT OF AN INCREASED CALCIUM ION CONCENTRATION ON ELECTRICAL AND CONTRACTILE ACTIVITY OF THE SMOOTH MUSCLES OF THE GUINEA PIG URETER

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Smooth muscle cells of the guinea pig ureter were studied by the double sucrose gap method. An increase in the calcium ion concentration in the Ringer–Locke solution to 22 mM led to hyperpolarization and to a slight increase in the resistance of the membrane. The amplitude of the first spike potential and the height of the plateau were lowered but the amplitude of the oscillations was increased. In sodium-free Ringer–Locke solution, when the action potential in the smooth muscle cells of the ureter was converted into a simple spike potential, a marked increase in amplitude of the action potential was observed with an increase in the calcium ion concentration.

KEY WORDS: smooth muscles; ureter; action potential; calcium ions.

The smooth muscle cells of the ureter (SMCU) of guinea pigs have a compound action potential (AP) which consists of the first spike potential and plateau on which oscillations are superposed. The AP plateau is observed only if sodium ions are present in the surrounding solution whereas the spike potential and the oscillations on the plateau disappear if calcium ions are removed [2, 4, 11].

It was accordingly decided to investigate the dependence of the spike potential and oscillations on the external calcium ion concentration and also to study the effect of an increase in the calcium ion concentration on the membrane resistance and contractile activity of the SMCU.

EXPERIMENTAL METHOD

Segments cut from the middle part of the guinea pig ureter, 15-20 mm long, were studied by the double sucrose gap method [1, 7]. To record electrical potentials and stimulate the muscle cells, chlorided silver electrodes were used. The muscles were stimulated by single square pulses from 1 to 3 μA in strength and 1-3 sec in duration. Contact between the electrodes and muscles was effected through agar bridges. The original Ringer–Locke (RL) solution had the following composition (in mmoles/liter double-distilled water): NaCl 154.0, $NaHCO_3$ 1.8, KCl 5.6, $CaCl_2$ 2.2, glucose 5.6. In the sodium-free RL solution the NaCl was replaced by sucrose and the $NaHCO_3$ by $KHCO_3$ (RLS solution). The calcium ion concentration in the surrounding solution was increased by the addition of the appropriate amount of calcium chloride to the RL and RLS solutions.

EXPERIMENTAL RESULTS

An increase in the calcium ion concentration in the RL solution to 5, 11, and 22 mM led to hyperpolarization of the membrane by 1-2 mV, a slight increase in the input resistance, and an increase in the threshold of excitation (Fig. 1, I). Hyperpolarization and the increase in membrane resistance following an increase in the calcium ion concentration also are observed in the annular smooth muscles of frogs [3]. Meanwhile, in experiments on the rat portal vein [6], an increase in the concentration of calcium ions leads to hyperpolariza-

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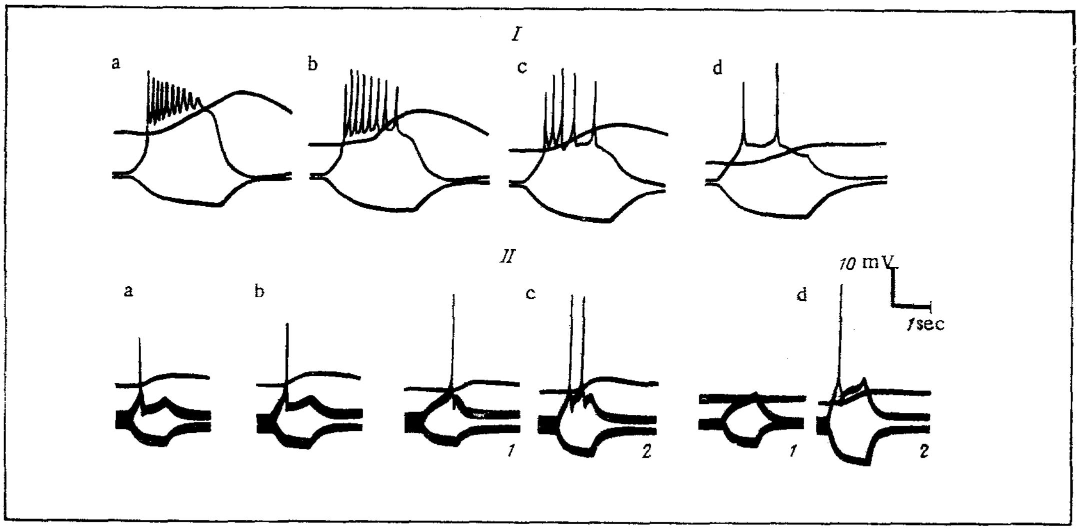

Fig. 1. AP, contraction, and electrotonic potentials (AET) in guinea pig SMCU in response to an increase in the calcium ion concentration in RL and RLS solutions. I: a) Above: catelectrotonus (CET), AP, and contraction of SMCU during action of depolarizing current in RL solution; below: anelectrotonus (AET) in response to action of hyperpolarizing current; b, c, d) the same, with increase in calcium ion concentration to 5, 11, and 22 mM, respectively. Strength of stimulating current everywhere the same. Here and in Figs. 2 and 3: upward deflection of beam, depolarization; downward, hyperpolarization. II: a) CET, AP, contraction, and AET of SMCU in RLS solution; b, c, d) the same, during increase in calcium ion concentration to 5, 11, and 22 mM, respectively. Strength of stimulating current in a, b, and c_1 the same; in c_2 and d_1 twice; and in d_2 three times as strong.

tion and to a decrease in the membrane resistance. This could indicate that with an increase in the calcium ion concentration mainly a decrease in the permeability to sodium ions is observed in the smooth muscles of the guinea pig ureter and the frog stomach, whereas in the rat portal vein there is an increase in permeability predominantly for potassium ions. Opposite results have been obtained on the guinea pig taenia coli. According to some observations [5], hyperpolarization in response to an increase in the calcium ion concentration is accompanied by an increase in the membrane resistance and is connected with a reduction in permeability for sodium ions, whereas other workers [8, 10] explain the membrane hyperpolarization by an increase in permeability for potassium ions.

The changes in AP of SMCU with an increase in the calcium ion concentration in the RL solution were of the following character: The amplitude of the first spike potential in most experiments was reduced compared with initially (Figs. 1, I and 3A). The amplitude of the AP plateau was reduced, whereas the amplitude of the oscillations was increased (Figs. 1, I and 3B). The amplitude of contraction during an increase in the calcium ion concentration in the RL solution depended on the number and frequency of oscillations on the AP plateau (Fig. 1, I).

It is interesting to note that after rinsing the SMCU with normal RL solution, when the calcium ion concentration in the surrounding solution fell sharply, an increase in the amplitude of the first spike potential and an increase of 2-2.5 times in the duration of the plateau were observed in the course of 2-3 min (Fig. 2, IIc).

The results of this series of experiments (the increase in amplitude of the oscillations, decrease in amplitude of the first spike potential, decrease in amplitude of the plateau) thus indicate that with an increase in the calcium ion concentration in RL solution complex changes take place in the shape of AP of the SMCU.

Meanwhile, in the smooth muscle cells of the taenia coli, which possess a simple spike AP, the amplitude of AP depends on the external calcium ion concentration [8-10]. In SMCU, AP also acquires the shape of simple spike AP on the removal of sodium ions from the surrounding solution [2, 4, 11].

In the next series of experiments the effect of an increased calcium ion concentration on AP generation by SMCU was therefore investigated in sodium-free solution (RLS). The removal of the sodium ions and most of the chlorine ions from the surrounding solution led to depolarization of the membrane by 8-10 mV, an increase in the membrane resistance of the SMCU, the appearance of spontaneous activity, and conversion of the complex AP of SMCU into a simple spike potential.

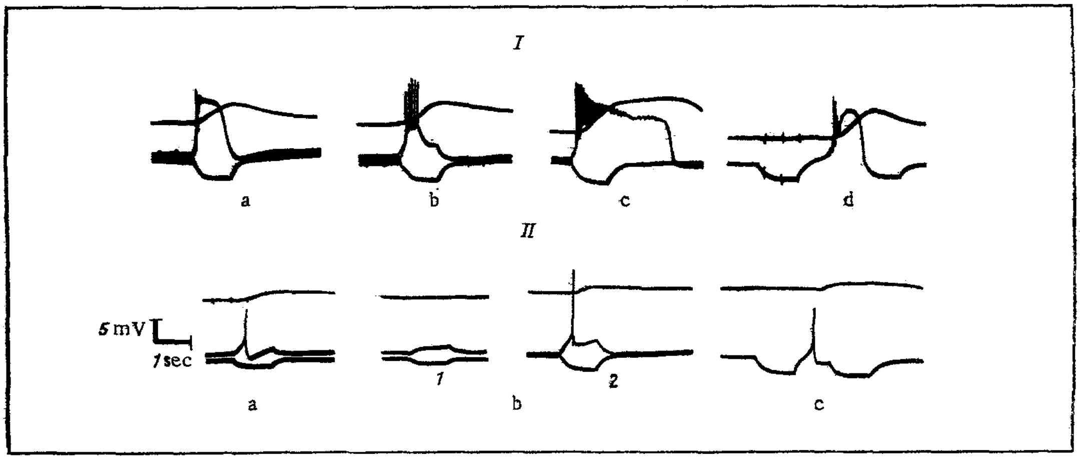

Fig. 2. AP, CET, and contraction of SMCU of guinea pig with increase in calcium ion concentration in RL and RLS solutions and after preliminary hyperpolarization of SMCU membrane. I: a) CET, AP, contraction, and AET of SMCU in RL solution; b) the same during exposure to Ca^{2+} in a concentration of 22 mM; c) the same, after rinsing with RL solution (2nd min); d) CET, AP, ', and contraction of SMCU in RL solution after preliminary membrane hyperpolarization. Strength of stimulating current in a, b, and c the same; in d doubled. B. II: a) CET, AP, contraction, and AET of SMCU in RLS solution; b) the same during exposure to Ca^{2+} in concentration of 22 mM; c) CET, AP, and contraction of SMCU in RLS solution after preliminary membrane hyperpolarization. Strength of stimulating current in a and b_1 the same; in b_2 and c doubled.

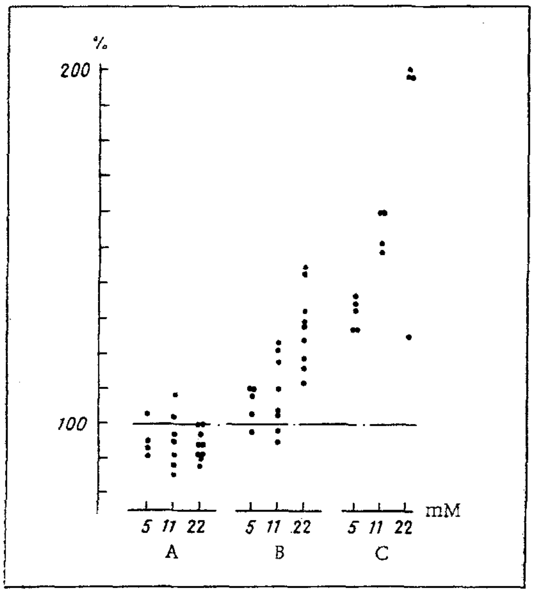

Fig. 3. Change in amplitude of AP of SMCU depending on calcium ion concentration in RL and RLS solution. A. Change in amplitude of first spike potential of SMCU with an increase in calcium ion concentration in RL solution. Horizontal line shows amplitude of first spike potential in RL solution with a normal calcium ion concentration (2.2 mM), taken as 100%. B. Change in amplitude of oscillations of SMCU with increase in calcium ion concentration in RL solution. Horizontal line shows amplitude of oscillations in RL solution with normal calcium ion concentration. C. Change in amplitude of AP of SMCU with increase in calcium ion concentration in RLS solution. Horizontal line shows amplitude of AP in RLS solution with normal calcium ion concentration. Abscissa, calcium ion concentration (in mM); ordinate, amplitude of AP (in %).

Increasing the calcium ion concentration in the RLS solution to 5.0, 11.0, and 22.0 mM was accompanied by membrane hyperpolarization of SMCU by 3-5 mV, a decrease in membrane resistance, an increase in the threshold of excitation, and a considerable increase in the amplitude of the spike potential (Fig. 1, II and Fig. 3C).

The decrease in the membrane resistance of SMCU with an increase in the calcium and chlorine ion concentrations in the RLS solution can evidently be explained by an increase in membrane conductivity as a result of the increase in the total ion concentration in the solution.

Despite the increase in amplitude of AP with an increase in the calcium ion concentration, the amplitude of contraction was unchanged (Fig. 1, Ia–c). Only with the appearance of secondary AP at catelectrotonus was an increase in the contractile response observed (Fig. 1, IIc_2).

Since an increase in the calcium ion concentration in the RL and RLS solutions was accompanied by hyperpolarization of the SMCU membrane, it was necessary to discover how membrane hyperpolarization itself affected the shape and amplitude of AP.

As Fig. 2, Id shows, in RL solution preliminary hyperpolarization of the SMCU membrane increases mainly the amplitude of the first spike potential and, to a lesser degree, the amplitude of the oscillations, and it also very slightly shortens the duration of the AP plateau and increases its amplitude. As was stated above and as will be seen in Fig. 2, Ib, the changes in AP with an increase in the calcium ion concentration in RL solution were of a different character.

In RLS solution preliminary hyperpolarization of the SMCU membrane increased the amplitude of AP by a much lesser degree than did the action of an increased calcium ion concentration (Fig. 2B, b_2, c).

Consequently, changes in AP of SMCU with an increase in the external calcium ion concentration are mainly connected with the specific action of the calcium and not with the membrane hyperpolarization.

The results of these experiments thus demonstrate that with an increase in the calcium ion concentration in normal Ringer–Locke solution complex relations are observed between the sodium, calcium, and, possibly, potassium conductivities of the membrane at the time of AP development in SMCU. Calcium ions entering the cell during AP development are known to activate the outward potassium current [12]. On the removal of sodium ions from the surrounding solution, when the AP in SMCU loses its plateau and is converted into a simple spike potential, an increase in amplitude of AP is observed with an increase in the external calcium concentration. However, for a final solution to the problem of the contribution of the various ion currents to electrogenesis of the AP of SMCU, voltage clamp experiments are necessary.

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