ROLE OF SODIUM AND POTASSIUM IONS IN ACTION POTENTIAL GENERATION IN THE SMOOTH MUSCLE CELLS OF THE URETER

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Evoked action potentials in the smooth muscles of the guinea pig ureter in Ringer-Locke solution consist of a first phase of fast depolarization accompanied by a phase of slow repolarization (a plateau). In sodium-free Ringer-Locke solution the resistance of the membrane is increased and spontaneous activity appears, in the form of simple spike potentials, which were recorded for 1-2 h, are similar in form. Consequently, whereas the first part of the action potential is independent of the external sodium ion concentration, and possibly arises on account of the entry of calcium ions into the cell, development of the plateau requires the presence of sodium ions. The duration and height of the plateau are regulated by calcium ions.

Action potentials in the circular smooth muscles of the frog's stomach [4], the smooth muscles of the taenia coli of the guinea pig [5], and of the portal vein of rats [3], when kept in sodium-free solution, can be evoked for 1-2 h. In sodium-free solution, moreover, the amplitude and steepness of rise of the action potentials were actually increased. At the same time, in the smooth muscles of the cat's ureter [6-8], on replacement of sodium ions by various substitutes, the amplitude and steepness of rise of the evoked action potentials were reduced, and after the preparation had been in the sodium-free solution for 30 min, no electrical activity could be evoked even by stimulation of maximal intensity. Hence, unlike in other smooth muscles, in the smooth muscles of the cat's ureter the action potential is dependent on the external sodium ion concentration.

It was therefore decided to investigate the role of sodium and calcium ions in action potential generation in the smooth muscles of the guinea pig's ureter and also to examine the effect of sodium and calcium ions on the permeability (resistance) of the membrane of these muscles.

EXPERIMENTAL METHOD

Experiments were carried out on smooth muscles of the guinea pig ureter by the method of a double "sucrose bridge" [1, 2]. A segment of the ureter without the proximal end, 15-20 mm long and 0.2-0.3 mm in diameter, was stretched by means of a 2-g weight and then fixed in the chamber of a double "sucrose bridge." Potentials were recorded by Ag–AgCl electrodes, and the object was simulated by a weak polarizing current through platinum electrodes. All the electrodes were connected to the corresponding segments of the muscle strip via the inflowing and outflowing solutions. The recording electrodes were connected to the input of a cathode follower, from which the signals were fed into the input of a type VÉKS-4M CRO. Parallel recordings of the potentials were made on photographic film by means of the camera attached to the CRO and by means of a type KSP 4 electronic automatic-writing potentiometer on oscillographic paper.

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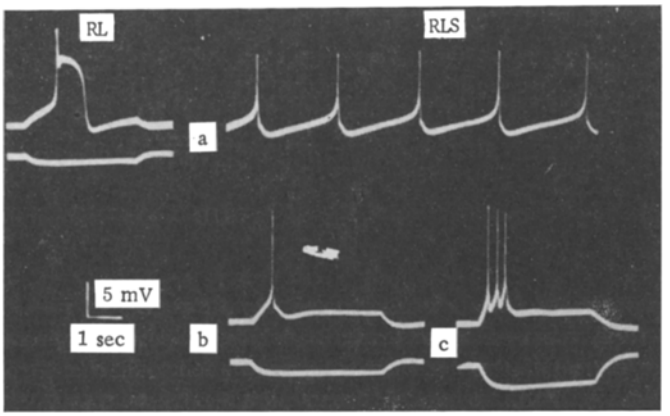

Fig. 1. Action potentials and electronic potentials in smooth muscles of the guinea pig ureter in normal and sodium-free RL solution. RL: above – catelectrotonous and action potential in smooth muscles of ureter during action of depolarizing current in normal RL solution; below – anelectrotonus during action of hyperpolarizing current ($2 \cdot 10^{-7}$ A). RLS: a) spontaneous action potentials in smooth muscles of ureter in RL solution in which sodium and chloride ions were replaced by sucrose (RLS solution); b, c) catelectrotonus and action potential (above) and anelectrotonus (below) in smooth muscles of ureter after being kept in RLS solution for 20 min. Current $1 \cdot 10^{-7}$ A (b) and $2 \cdot 10^{-7}$ A (c). Here and in Figs. 2 and 3, upward deviation of beam denotes depolarization, downward denotes hyperpolarization.

The following solutions were used for the investigation: 1) Ringer–Locke (RL) solution containing (in mmoles/liter): Na^+ 155.8, K^+ 5.6, Ca^{++} 161.8, HCO_3^- 1.8, and glucose 5.6; 2) RL solution with an increased concentration of calcium ions (22 mM); 3) RL solution in which the sodium and chloride ions were replaced by sucrose (RLS); 4) RLS solution with an increased concentration of calcium ions (22 mM). Before being supplied to the sucrose bridge chamber the RL solution was aerated with a mixture consisting of 95% O_2 and 5% CO_2 and heated to a temperature of 33-34°C.

EXPERIMENTAL RESULTS

The smooth muscles of the guinea pig ureter, from which the proximal end containing the pacemaker had been removed, possessed no spontaneous activity at a temperature of 33-34°C. As Fig. 1 shows, the action of the cathode of the polarizing current on the smooth muscles of the ureter in RL solution initially caused the appearance of a prepotential, which subsequently changed, on reaching the critical level of depolarization of the membrane, into an action potential (AP). The AP consisted of a fast phase of depolarization, 10-15 mV in amplitude, accompanied by a slow phase of repolarization (a plateau) lasting 1-3 sec. At the end of the AP, a well-marked after-hyperpolarization developed. Disconnecting the polarizing current was followed by disappearance of the catelectrotonus. Sometimes extra oscillations appeared on the plateau, but as a rule they were more clearly seen in Krebs's solution than in RL solution. In their shape, the APs recorded in the smooth muscles of the guinea pig ureter in these experiments by the double sucrose bridge method resembled APs in the smooth muscles of the guinea pig ureter recorded by a microelectrode technique, but they were of lower amplitude and longer duration [9, 11].

When the sodium and chloride ions in the RL solution were replaced by sucrose to begin with, hyperpolarization of the membrane was observed, and this was followed by gradual depolarization. On the average, 5-10 min after the beginning of exposure to the RLS solution, spontaneous activity appeared in the smooth muscles of the ureter. It consisted either of simple spike APs with prepotentials (Figs. 1, RLS, a) or of a slow depolarization wave with several spike APs superposed upon it. The spontaneous activity

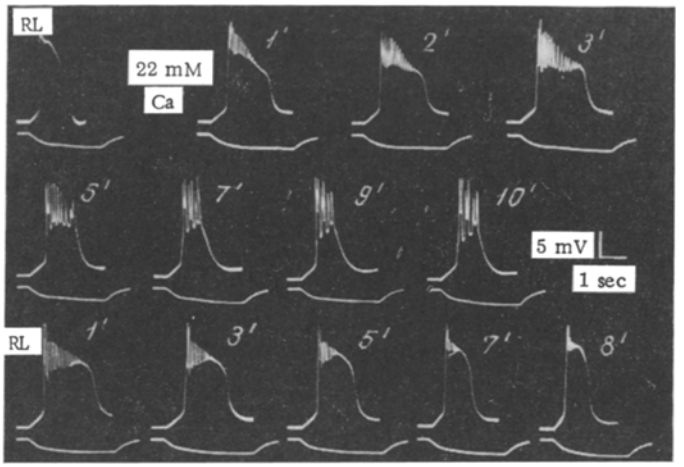

Fig. 2. Action potentials and anelectronic potentials in smooth muscles of the guinea pig ureter in RL solution with normal and increased calcium ion concentrations. RL – action potential during action of depolarizing current and anelectrotonus in smooth muscles of ureter in RL solution with normal calcium ion concentration (2.2 mM). After the inscription 22 mM Ca – the same, after exposure for 1, 2, 3, 5, 7, 9, and 10 min to RL solution with increased calcium ion concentration. After the inscription RL in the third row – the same, after rinsing for 1, 3, 5, 7, and 8 min with RL with normal calcium ion concentration. Current strength everywhere $2 \cdot 10^{-7}$ A.

continued for 20-60 min. Evoked APs could be recorded for 1-2 h. The anelectrotonus increased during continued exposure to the RLS solution, and in most experiments an anode off-response appeared. Usually the anelectrotonus reached a constant level after 15-20 min. The magnitude of the anelectrotonus in RLS solution was 3-4 times greater than in ordinary RL solution. Evoked APs in RLS solution, like spontaneous APs, resembled simple spike potentials, and their amplitude and steepness of rise were greater than in RL solution. Anelectrotonus and APs in response to stimulation by the cathode of a steady current in RL solution are shown in Fig. 1 on the left side, and in RLS solution on the right. As the duration of the latent period shows, the excitability of the smooth muscles of the ureter was increased in the latter case.

The most characteristic feature in the changes affecting the APs in RLS solution was thus disappearance of the plateau and conversion of the complex AP into a simple spike potential. The same results were obtained when sodium ions in the RL solution were replaced by choline ions. Consequently, for a plateau to develop, sodium ions must be present, whereas the first, fast phase of depolarization can also take place in the absence of sodium ions.

In RL solution with a normal concentration of sodium ions the duration of the plateau can be increased by increasing the concentration of calcium ions. As Fig. 2 shows, during the first 3-4 min of action of an increased calcium ion concentration, the duration of the plateau was doubled and extra oscillations appeared upon it. However, during the further action of calcium, the duration and height of the plateau were considerably reduced. The amplitude of the spike potentials, on the other hand, continued to increase. When the strip was rinsed with normal RL solution, the same picture was observed but the opposite order, namely: before the AP assumed its usual form, APs with a prolonged plateau with extra oscillations superposed upon it were observed. Calcium ions, at the beginning of their action and during rinsing, thus lead to an increase in the duration of the plateau in the presence of sodium ions. During the more prolonged action of calcium ions the plateau is reduced in size and is converted into a slow wave with large spike action potentials superposed upon it.

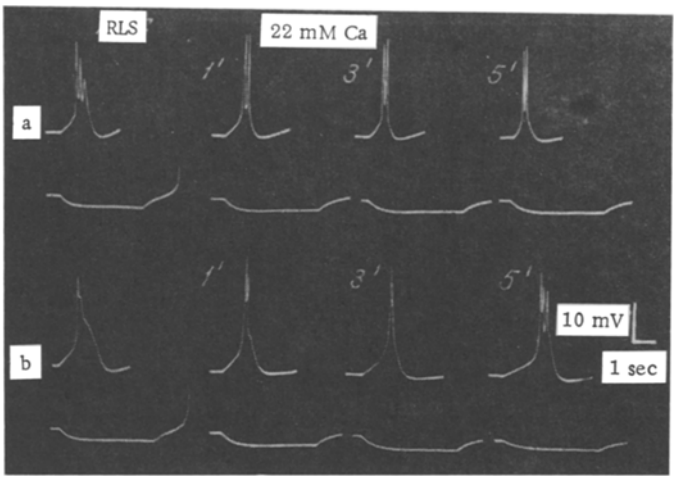

Fig. 3. Action potentials and anelectrotonic potentials in smooth muscles of the guinea pig ureter in sodium-free RL solution with normal and increased calcium ion concentration. RLS: a) action potential during the action of an depolarizing current and anelectrotonus in smooth muscles of the ureter after 30 min in RL solution in which the sodium and chloride ions were replaced by sucrose (RLS solution) and with an increase in the calcium ion concentration in RLS solution (22 mM Ca; action for 1, 3, and 5 min); b) the same after the action of RLS solution for 45 min. Current strength everywhere $2 \cdot 10^{-7}$ A.

Anelectrotonus at the beginning of action of an increased calcium ion concentration was very slightly increased. During the more prolonged action of calcium ions, a tendency was observed for the anelectrotonus to decrease.

The action of an increased calcium ion concentration in RLS solution not containing sodium ions differed from its action in the presence of sodium ions. Calcium ions in sodium free solution increased only the amplitude (Fig. 3a) and number of the spike potentials (Fig. 3b). The same effect was shown by calcium ions in the presence of RL solution in which the sodium ions had been replaced by choline. The decrease in anelectrotonus during the action of calcium in sodium-free RLS solution was more marked (Fig. 3a, b) than in normal RL solution (Fig. 2). Under the influence of calcium ions, disappearance of the anode off-response was observed (Fig. 3a, b). The decrease in anelectrotonus in RLS solution was probably due to an increase in the permeability of the membrane to potassium ions or to the calcium ions introduced.

Hence, in the sodium-free solution, evoked APs were observed in the smooth muscles of the guinea pig ureter for 1-2 h. The shape of the APs was altered in sodium-free solution: complex APs, consisting of a phase of fast depolarization and a plateau, were converted in simple spike action potentials. Consequently, whereas the first part of the AP – the phase of fast depolarization – is independent of the external sodium ion concentration, and it possibly arises largely through the entrance of calcium ions into the cell, the presence of sodium ions is essential for development of the plateau. The duration and height of the plateau are regulated by the ratio between the concentrations of sodium and calcium ions. Similar results were obtained by Kuriyama and Tomita [10] in a recently published investigation.

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