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ORIGINALS

The Influence of Calcium on the Electrical and Mechanical Activity of the Guinea Pig Ureter

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Summary. Low calcium concentration in the external medium depolarises the membrane of smooth muscle cells of the ureter and their excitability diminishes. The Ca^{++}dependent oscillations of the action potential disappears, while the plateau component is more resistent. Analogous reactions are observed by the addition of Ca^{++} antagonists such as Lantanum or Verapramil. A high Calcium concentration produces a slight hyperpolarisation, which stabilises the membrane and enhances the spike component while decreasing the plateau component of the action potential. It is possible that several drugs act indirectly on the ureter by changing the relationship between the calcium concentration of the external versus the internal medium.

Key words: Calcium ions, Action potential, Ureter, Membrane potential.

In previous papers we have suggested that many drugs (e. g. antibiotics (10), catecholamines (11)) may act in a direct way on the smooth muscle cells of the ureter. A possible site of interference of the drugs is the ionic equilibrium of the cell membrane during rest and the ionic exchanges of intra- and extracellular medium during activity.

The processes involved in the excitation-contraction coupling may be summarised as follows (8): excitation depolarises the cell membrane and changes its permeability to various ions; there is an influx of sodium and calcium, while potassium flows out of the cell. The entering Ca^{++}, together with the Ca^{++} released from the sarcoplasmic reticulum or the inside of the membrane, increase the concentration of intracellular free Ca^{++}, thereby activating the actomyosine complex. In this paper the influence of Calcium ions on the electrical and mechanical activity of the ureter are reported.

Material and Methods

20 guinea pigs of both sexes weighing between 150 and 350 g were used. They were killed by a blow on the head and bled. The ureter was dissected free from adventitial tissues. All these procedures were performed taking care to moisten the tissues continually with Krebs solution at room temperature.

A ureteric segment 3 to 6 cm in length was mounted in an organ-bath with a capacity of 5 ml. The fluid in the bath was supplied from a large reservoir at a constant flow rate of 4 ml/min and renewed by overflow. The fluid in the reservoir and bath were held at a constant temperature of 37° C by a Lauda thermostat. By switching from the Krebs reservoir solution to a parallel reservoir containing test solutions or drugs, the extracellular medium could be rapidly changed.

A hollow glass tube of 0,9 mm outer and 0,4 mm inner diameter was inserted into the distal end of the ureter and connected via a small plastic catheter to a pressure transducer. The glass tube also served as a support for the microelectrodes. The microelectrodes consist of glass micropipettes filled with 3 M. KCl., and loosely suspended in a Ag-agarbridge so that they are not dislocated during contractile movements of the ureter. The microelectrodes are inserted in the neighbourhood of a cell by slightly turning the micrometer screw which holds the bridge; penetration into the cell is

then obtained by a little tap on the table. The membrane and action potentials were displayed on an oscilloscope via a high impedance input amplifier simultaneously with the pressure recordings, and the traces were recorded on film. Apart from the microelectrode, several bipolar recordings with external macroelectrodes were made.

In the control (normal) conditions Krebs-Ringer solutions of the following composition (mM) were used: NaCl 137; KCl 5,9; $CaCl_2$ 2,5; $MgCl_2$ 1,2; $NaHCO_3$ 15,5; NaH_2PO_4 1,2; glucose 11,5. The solution is aerated with a gas mixture of 95% O_2 and 5% CO_2. In Ca^{++} free Krebs solution $CaCl_2$ was replaced by an equivalent amount of NaCl; the changes of the concentrations of Na^+ and Cl^- in this solution are so small that the observed reactions of the tissue may be attributed to the absence of Ca^{++}. It should be noted that this solution is different from the zero-Calcium solution ($< 10^{-2}$ mM) containing glycol-ether-diaminetetraacetic acid (Triggle 1971), which easily causes irreversible damage of the tissue. High external Ca^{++} medium (10 mM Ca^{++}) was obtained by replacing the $CaCl_2$ of normal Krebs solution by a mixture of 1 portion $CaCl_2$ (3g/250 ml) and 3 portions Ca lactate (25g/250 ml).

In the experiments with Lanthanum nitrate, the bicarbonate and phosphate buffer of normal Krebs are replaced by Hepes buffer at pH 7 because La^{+++} precipitates in an alkaline solution and in the presence of phosphate and bicarbonate. This solution is aerated with oxygen.

D 600 is a methoxy-derivate of verapramil, of which the calcium antagonistic properties have been demonstrated by Fleckenstein et al. (5)

Results

The normal intracellular action potential of the guinea pig ureter has been described by several authors (12, 7). It consists of a plateau of depolarisation of about 500 msec. duration with 5 to 10 oscillations superimposed on the initial part of the plateau. The normal membrane potential is about 60 mV.

1. Ca^{++} free medium

Within the first ten minutes of incubation in the Ca^{++} free medium, the ureteric muscle cell membrane depolarises by about 16 mV and the action potential is shortened (Fig. 1); the number and amplitude of the oscillations rapidly decrease and the spikes soon disappear completely. The rate of rise is decreased and the plateau is elevated. Returning to normal Krebs solution rapidly restores the oscillations and the rate of rise of the action potential.

Extracellular recording at different points along the ureter (Fig. 2) provide a clearer picture of the alterations of its excitability. Within the first six minutes in the calcium-free medium, all spontaneous activity is abolished. A transient increase in activity, as observed in many other excitable tissues (2) was never seen. However electrical stimulations may still evoke a response during the first hour in the Ca^{++} free medium, but the stimulus intensity must be gradually increased and the responses become more and more delayed. The conduction velocity decreases and the conduction distance also decreases so that finally only a small potential change is observed at the electrodes nearest to the site of the stimulus. Returning to normal Krebs solution restores the oscillations and the shape of the action potential almost immediately but the conduction velocity returns to its original value more slowly. The amplitude of the ureteric contraction diminishes parallel to the changes of the action potentials; it becomes normal again on returning to normal Krebs solution.

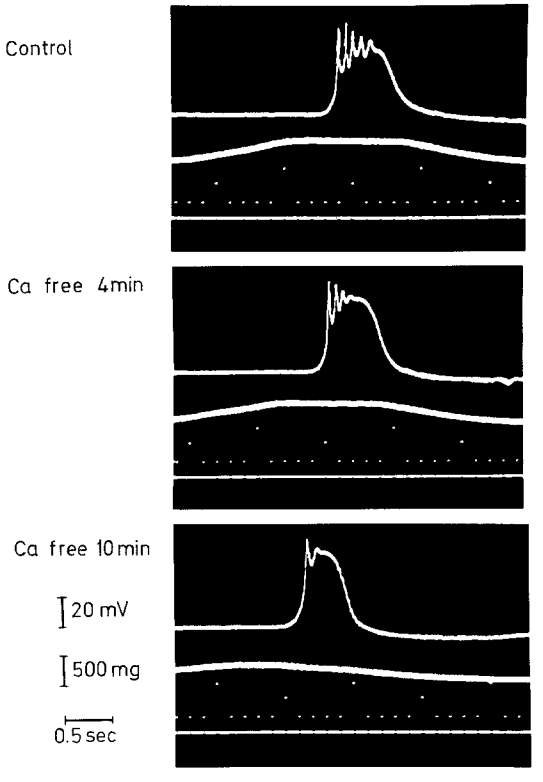

Fig. 1. Modification of the action potential and intraluminal pressure in the guinea pig ureter during exposure to calcium-free solution

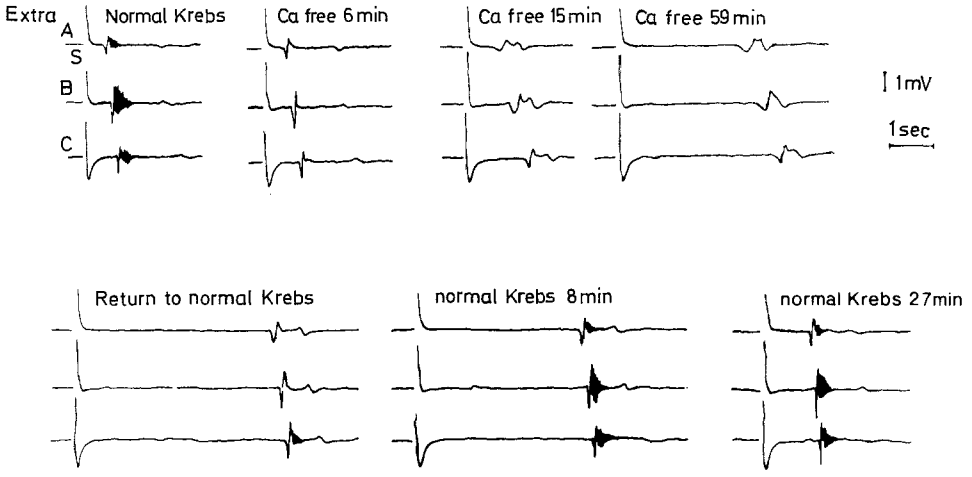

Fig. 2. Influence of 1 h of Ca^{++} free Krebs solution on the action potential in the guinea pig ureter. The extracellular recordings are obtained at 21.4 (A), 28.8 (B) and 33.7 (C) mm from the stimulus electrode

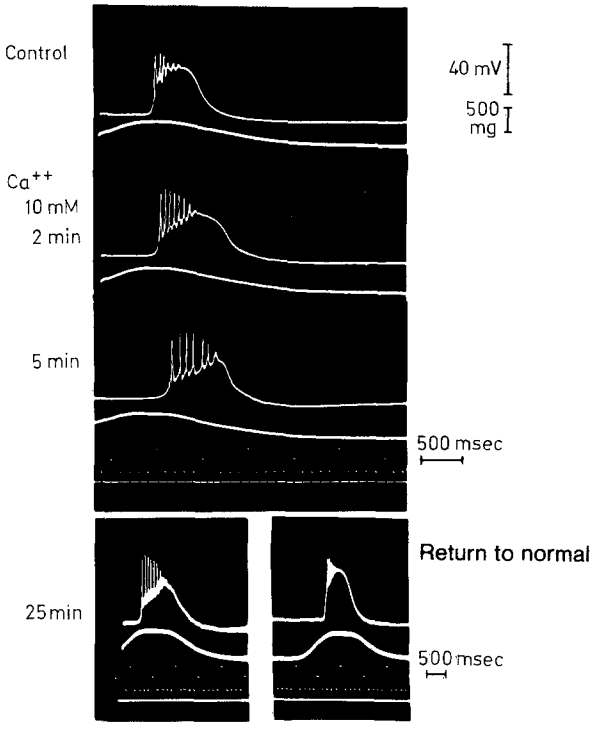

Fig. 3. Influence of high Calcium medium (10 mM) on the intracellular action potential and tension of the guinea pig ureter

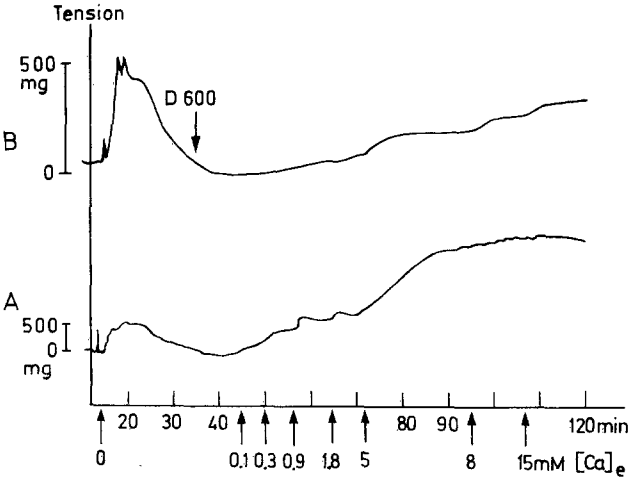

Fig. 4. Lower curve (A): influence of concentrations of Ca^{++} on the ureteric contraction, induced by 80 mM K^+ - Krebs (at the first arrow). Upper curve (B): inhibitory action of D600 (1 mg/l) on this process. Dog ureter

2. High calcium medium (up to 10 mM Ca^{++})

In a high calcium medium the cell membrane of the guinea pig ureter hyperpolarises by about 8 mV. The rate of rise of the action potential is increased as well as the number, amplitude and interval of the superimposed spikes (Fig. 3). The amplitude of the plateau is greatly diminished and may disappear completely. The threshold for electrical stimulation is increased. The tension development is greater but no tonic contracture appears.

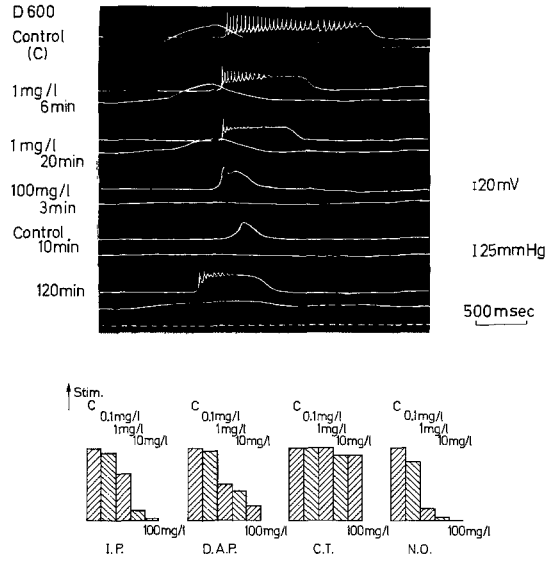

Fig. 5. Effect of D600 (derivative of Verapramil) on the action potential and intraluminal pressure in the guinea pig ureter. The lower diagrams give in percentages the changes of intraluminal pressure (I.P.), duration (D.A.P.), conduction time (C.T.) and number of initial oscillations (N.O.) of the action potentials at different concentrations of D600. The percentages are calculated from the mean of 7 values. R. Control = return to normal Krebs solution

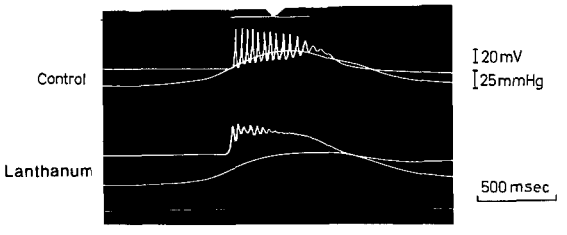

Fig. 6. Influence of 5×10^{-5} M lanthanum on the action potential and intraluminal pressure of the guinea pig ureter

3. Influence of the external calcium concentration on the K^+ induced contracture

As will be shown in another publication a high external K^+ concentration of 80 mM provokes a tonic contraction of the ureter which is not accompanied by action potentials. It is found that the amplitude of this contraction is calcium-dependent; it increases with increasing (Ca^{++}) between 0 and 10 mM and no contraction occurs if external Ca^{++} is absent. In Fig. 4 the same phenomena are shown to occur in the dog ureter.

4. D600 (methoxy derivative of verapramil)

The total duration of the action potential, the number of spikes, the excitability, the amplitude of the first spike and the maximum rate of rise of the action potential in the guinea pig ureter are all decreased by concentrations of D600 above 1 mg/1. The plateau is increased at lower and becomes flat at higher concentrations. The contraction amplitude is reduced. At a concentration of 100 mg./1. D600 all electrical and mechanical activity disappears. The return to normal in normal Krebs solution is very slow and after 20 minutes the contraction has regained only half of its previous amplitude (Fig. 5). In K-depolarised ureters D600 induced a relaxation. These effects of D600 are increased in low calcium medium and reduced by high external calcium conditions.

5. Lanthanum

5×10^{-3} mM La $(NO_3)_3$ added to a Hepes-Krebs solution which contains 1.25 mM Ca^{++} (see methodology) has only a transient influence on the guinea pig ureter. At 10^{-2} mM the rate of rise of the action potential and the ocillations become markedly reduced (Fig. 6); the amplitude of the contractions rapidly declines. At 5×10^{-2} mM all electrical and mechanical activity stops and the ureter is refractory to electrical stimulation. This quiescence of the cells persists for at least 30 min after returning to normal Krebs solution.

Discussion

As in other tissues (9) Ca^{++} exerts a stabilising action on the ureteric membrane manifested by the hyperpolarisation, the reduced spontaneous firing, the increased threshold for external stimuli and the increased interval between the oscillations of the action potential. Kobayashi (7) demonstrated that in the cat ureter, where the action potential does not

show oscillations, the increased (Ca^{++}) also caused a membrane hyperpolarisation and an increased resistance to electrical stimulation. In calcium free solution the membrane is depolarised by about 18 mV.

The influence of calcium on the action potential of the guinea pig ureter is different on the spike component and on the plateau component. In low calcium and in the presence of Ca^{++} antagonists such as D600 and La^{+++}, the number and amplitude of the oscillations of the action potential are greatly reduced. A conversion of the combined spike-plateau configuration into a pure plateau is observed in calcium free media and the amplitude of this plateau is increased. In calcium excess however the oscillations are numerous and their amplitude is increased, while the amplitude of the plateau component becomes very low. Bennett et al. (7) have even reported single spikes at very high calcium concentrations. It may therefore be assumed that the spikes of the action potentials are dependent on the presence of calcium.

The slow loss of contractility in Ca^{++} free medium (45 min) and the almost immediate recovery of the response to electrical stimuli on returning to normal calcium concentration suggest that the intracellular free calcium ions, necessary for contraction, arise not only from the entry of external Ca^{++} but also from the release of Ca^{++} sequestered on the inside of the membrane or from the sarcoplasm. Analogous rates of disappearance and recovery of response to drugs or electrical stimulation in Ca^{++}-free media are reported for the uterus (Daniel et al. 4) and the taenia coli (3).

The doses of D600 required to reduce the ureteric contractions is about a hundred times higher than that required to reduce uterine contractions (5). Relaxation of the ureter by verapramil has also been observed by Golenhofen and Hannappel (6).

As in other tissues the contraction induced in high K^+ medium is dependent on the external Ca^{++} concentration: it is diminished in a low calcium medium and by the addition of D600.

Calcium concentrations may play an important role in the effects of various drugs on the ureter. We have observed (11) for instance that the ureteral excitation produced by adrenaline can be reinforced by addition of Calcium in the external medium. Further study is required to determine if pathological conditions in the blood calcium levels may have practical consequences on ureteral peristalsis.

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