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The Influence of Sodium on the Electrical and Mechanical Activity of the Ureter

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Summary. In Sodium-deficient solutions both the electrical and mechanical activity of the ureter are reduced. The plateau component of the action potential in the smooth muscle cell of the guinea pig ureter is more affected than the oscillations. Tetrodotoxin, which blocks the action potential in nerves, does not influence activity or conduction in the ureter. This is an important argument for the myogenic conduction of activity in this tissue.

Key words: Ureter, Sodium, Electrical activity of smooth muscles.

Although the Ernst and the Donnan equations, which describe the relation between the external and internal concentrations of Na^+, K^+ and Cl^- are valuable in most tissues, it is known that in some smooth muscles (such as the taenia coli) physiological findings argue against the application of the Na^+ hypothesis for the spontaneous generation of action potentials. We demonstrated in a previous paper that calcium plays an important role in the excitability of the guinea pig ureter (7). The present investigations were performed in order to determine the influence of Na^+ on the generation and form of action potentials and in the conduction of excitation in the ureter.

Material and Methods

Twenty guinea pig ureters were examined in vitro. The experimental procedure has been described extensively in a previous paper (6). Intracellular potentials are recorded by glass microelectrodes filled with 3 M KCl and connected to a cathode follower preamplifier with capacitance compensation. In preparations which were not spontaneously active, action potentials were induced by means of electrical stimulations applied to one end of the preparation at intervals of 1 to 3 min. Potential changes were displayed on a cathode-ray oscilloscope screen and photographed. Isometric contractions in a longitudinal direction were recorded with a potentiometric pen-recorder.

The isolated ureters were mounted in an organ bath, continuously perfused by either Krebs or test solution at 37° C. The Krebs solution had the following composition (mM): 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 was aerated by a gas mixture of 95 % O_2 and 5 % CO_2 and its pH was 7.35.

The role of external sodium in the physiological activity of the guinea pig ureter was investigated by two procedures:

a) The activity of the ureter was studied in sodium deficient solutions. For this purpose the reservoir containing the Krebs solution for continuous perfusion of the organ bath was switched off and the organ bath was perfused by one of the following test solutions: 1) NaCl was replaced with equimolar tris [tris (hydroxymethyl) aminomethane] chloride; pH was adjusted to 7.4 by titration with HCl; NaH_2Po_4 and $NaHCO_3$ were omitted; the solution is aerated by O_2 only. 2) NaCl was replaced by the same amount of a sucrose solution of 100 g per liter

of water (154 mM NaCl is isosmotic with 292 mM sucrose) or of a choline-chloride solution of 21.5 g/l; in both solutions the sodium of the bicarbonate and phosphate buffers were not replaced. 3) In some experiments NaCl was replaced by an equivalent amount of LiCl; thus the chloride content remained constant while the sodium concentration was reduced.

b) the effect of Tetrodotoxin (TTX) was studied. This substance specifically blocks Na permeability in some tissues (1, 2).

Results

The normal membrane potential in the guinea pig ureter is between 56 and 60 mV. The action potential consists of a sustained depolarisation of about 500 msec duration and a series of 5 to 10 spikes are superimposed on the initial part of this plateau. At each normal contraction the tension developed is between 300 and 500 mg, and the intraluminal pressure between 20 and 40 mm Hg. Only preparations of the ureter which remain connected to the renal pelvis show spontaneous activity. The values of these parameters change in low sodium solutions.

1. NaCl-substitution with Tris-chloride

The replacement of sodium by Tris-chloride causes a depolarisation of the membrane by about 8 mV. Almost immediately after exposure to this solution, the duration and the amplitude of the plateau of the action potential shortens and the number and frequency of the oscillations decrease (Fig. 1). Within a few minutes all spontaneous activity is abolished. For another few minutes an electrical stimulation can still elicit a response but with an increasing latent period. This response consists of a single action potential with reduced amplitude and reduced rate of rise and fall. Its propagation distance is reduced. Some minutes after returning to normal Krebs solution, electrical excitability and normal configuration of the action potentials return.

2. NaCl-substitution with sucrose solution

Extracellular recordings of the action potential show that substitution of NaCl with sucrose causes spontaneous spike discharge of very high frequency and that conduction of the electrical activity is maintained. This spontaneous activity causes a tetanic contraction of the ureter, on which fluctuations of small amplitude and of a frequency similar to that of the spikes are superimposed. Such spontaneous activity may persist for more than two hours. The frequency of the spikes gradually diminishes

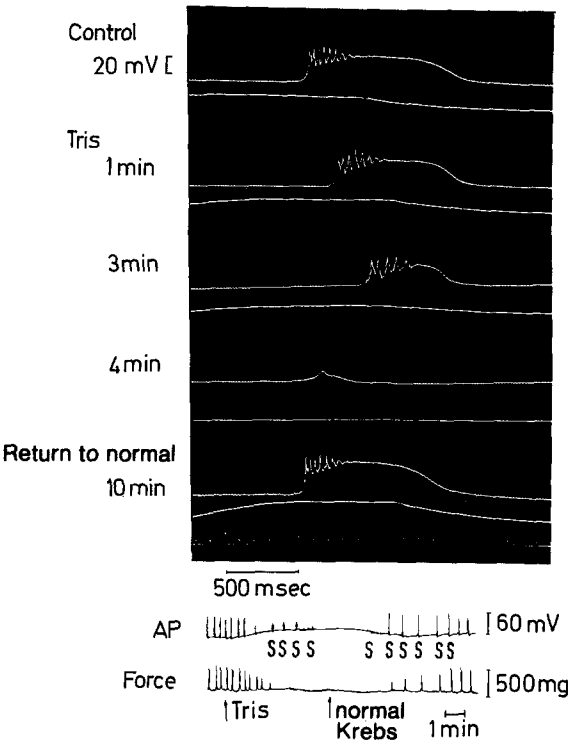

Fig. 1. Influence of substitution of Trischloride for sodium on the action potential and force development of the guinea pig ureter. S = stimulus (30 V, 100 msec)

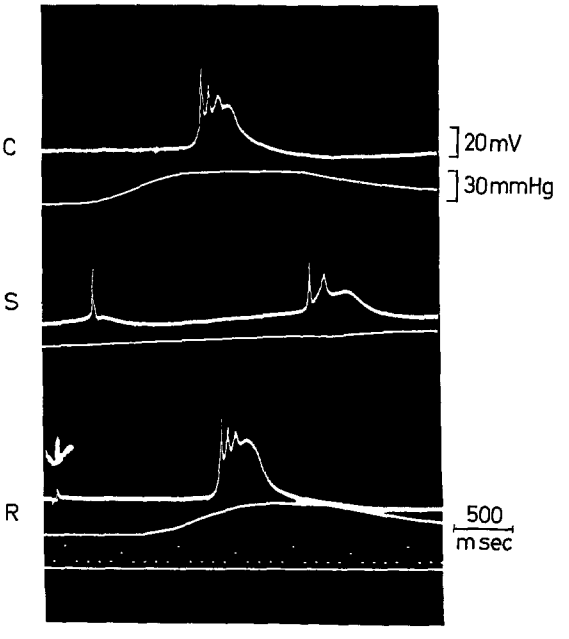

Fig. 2. Influence of substitution of sucrose (100 g. sucrose/1.) for NaCl on the intracellular action potential and intraluminal pressure development of the guinea pig ureter. C = control; S = sucrose substitution; R = return to normal Krebs solution

and signs of conduction block occur. Later on, the tissue becomes quiescent and inexcitable.

Intracellular recordings of the action potentials confirm that the normal plateau with initial oscillations is replaced by a single spike of reduced amplitude but at high frequency (Fig. 2). In some cells no spikes but only small oscillations of the potential were observed. Returning to Krebs solution normalises the latent period of the electrical stimulation and the spike configuration (plateau with oscillations) and the plateau may even be a little prolonged. The resting potential is decreased by about 2 to 4 mV only.

3. Substitution of choline-chloride for NaCl

Replacing NaCl by choline-chloride provokes, after a transient and short inhibition of the spontaneous activity, contractions of high frequency, but after each contraction the ureter relaxes completely. A study of the action potentials shows that the plateau and the frequency of the superimposed spikes diminish so that only single or double spikes of reduced and variable amplitude remain. (Fig. 3). These spikes may occur in irregular groups. Sometimes the single spikes are preceeded by slow potential oscillations of increasing amplitude with the form of pre-potentials (Fig. 4). After returning to normal Krebs solution the configuration of the action potential and the frequency of spontaneous contractions become normal.

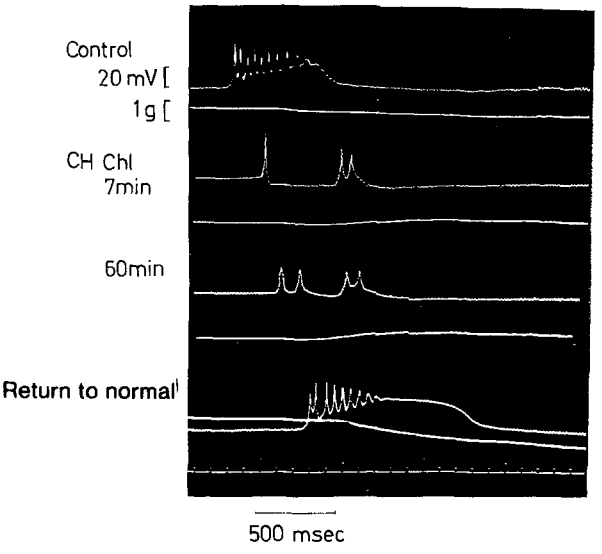

Fig. 3. Influence of substitution of choline-chloride (with 10^{-4} M atropine) (CH-Chl) for sodium on the guinea pig ureter. Upper trace: intracellular action potential; lower trace: tension

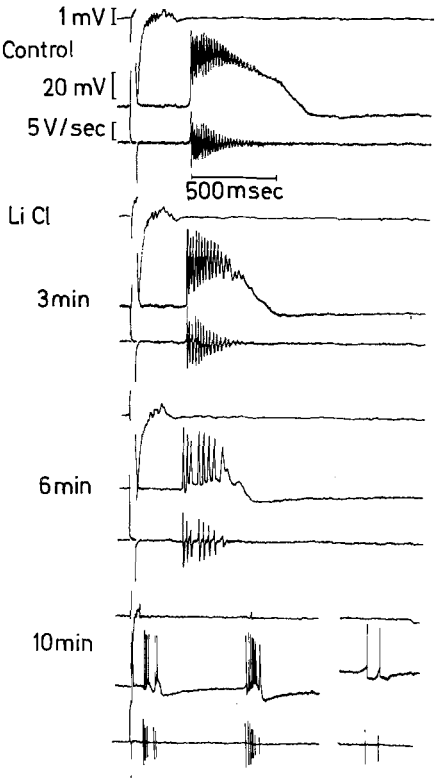

Fig. 5. Influence of substitution of lithium chloride for sodium on the extracellular action potential, the intracellular action potential and its rate of rise. Guinea pig ureter

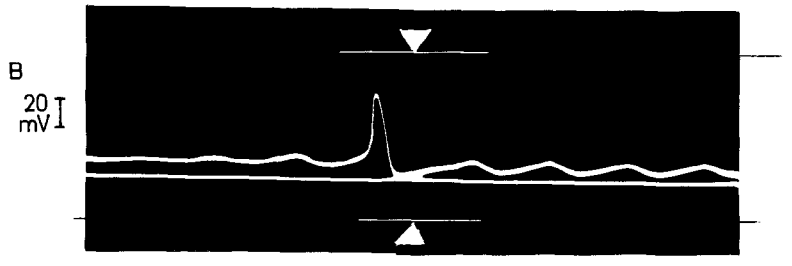

Fig. 4. Guinea pig ureter in choline-chloride-substituted Krebs solution (intracellular recording). Slow potential changes of increasing amplitude resulting in the generation of a single spike

4. Substitution of lithium chloride for NaCl

After substituting sodium by lithium the plateau component of the action potential disappears; the spike intervals increase until only single spontaneous spikes or irregular groups of fast single spikes persist. At first the amplitude and rate of rise of the spikes increase but later on both parameters diminish (Fig. 5). The membrane depolarizes by about 20 mV and the overshoot disappears after 5 min. The contractions are also greatly reduced.

5. Effects of tetrodotoxin (TTX) (10^{-9} to 10^{-4} M).

Addition of TTX in concentrations as high as 10^{-4} M to normal Krebs solution does not affect the resting potential, nor the action potential of the spontaneous contractions. The contractions induced by transmural slow electrical stimuli (single pulses of 10 msec) are not changed. However the contractions induced by electrical stimuli consisting of trains of pulses of 500 msec at 50/sec during 1 second are slightly reduced.

Discussion

The current experiments show that different sodium substitutes all cause similar changes of the action potential: the plateau component and the superimposed spikes completely disappear and are replaced by single spikes or bursts of single spikes. A possible explanation for this finding is that the plateau component and part of the initial spike is dependent on the external sodium. One should be aware however that any change of the ionic environment induces a chain of reactions, which make its effects more complex to analyse. For instance sodium substitution in the guinea pig ureter is accompanied by an intracellular decrease in potassium concentration from 159 mM to 108 mM with Tris-substitution and to 77 mM in Li^+ substitution (9).

In the cat ureter Kobayashi and Irisawa (3) found a shortening of the action potential in tris-chloride but a prolongation in choline-chloride and sucrose solutions. This prolongation in choline-chloride could be due to an acetylcholine effect (independent of nervous activity) and in the sucrose solution to the Cl^- withdrawal. The authors could therefore not settle the question whether sodium deficiency causes a shortening or a prolongation of the action potential in the ureter. Our results in the guinea pig ureter clearly demonstrate that Na^+ deficiency shortens the action potential, whatever the substitute.

After a period of spontaneous activity (lasting from a few minutes up to two hours according to the substitute) the excitability of the ureter completely disappears, although the membrane potential is maintained at a slightly depolarised level (55 mV in Tris, 40 mV in lithium). This is probably due to some alteration of membrane function. It is important to stress that 2.5 mM calcium is not sufficient to maintain the excitability of the guinea pig ureter. However in the cat ureter Kobayashi and Irisawa (3) demonstrated that an excess of calcium may restore the decreased activity in a low Na^+ medium. This might be due to a competitive action of Ca^{++} and Na^+ in the action potential. Kuriyama and Tomita (4) investigated the guinea pig ureter by the double sucrose gap method and came to the conclusion that the plateau of the action potential is probably due to an increase in the Na^+ conductance of the membrane, while the spikes are due to Ca^{++} entry. They postulated that Na^+ and Ca^{++} effects involve different parts of the cell membrane. One may, however, also invoke the hypothesis that Na^+ acts through a slow sodium channel, at which calcium acts competitively. Indeed, the action potential is not blocked by TTX, lithium substitutes for sodium in the action potential, adrenaline activates the action potential (6), and Manganese blocks the action potential according to Washizu (8). These four characteristics of the guinea pig ureter action potential correspond to the criteria which Rougier et al. (5) have postulated as characteristics of a slow Na^+ - Ca^{++} channel in heart muscle. It is known that the ureter has many properties analogous to that of heart muscle (10). Investigations of the currents during the action potential under different conditions are required before a similar system can be considered in smooth muscle.

Hille (1) reviewed the arguments which lead to the assumption that TTX selectively blocks the Sodium (or fast) channel in nerve membranes. It is unlikely that smooth muscle membranes are comparable to axon membranes since many smooth muscles show action potentials which are Ca^{++} dependent and which are not affected by TTX. The plateau component of the action potential in the ureter is shown to be Na^+ dependent, although TTX has no influence on it. Pharmacological studies under voltage clamp conditions are required for adequate interpretation of these facts.

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