Factors Controlling the Intracellular Concentration of Calcium and the Spontaneous Activity of the Ureter

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Abstract. The role of the electrogenic Na^+—Ca^{2+}-exchange mechanism in regulating the spike activity of the ureter was studied. The ureter cells were shown to be capable of generating action potentials (AP) in sodium-free Krebs solution. The time during which the spikes are generated is in exponential dependence on the concentration of calcium ions in the medium, $[Ca^{2+}]_o$ within 2.5 to 15 mmol/l. Simultaneously with the generation of the spikes, accumulation of calcium in the muscles is observed, proportional to the increase of $[Ca^{2+}]_o$. The addition of as little as 20 mmol/l Na^+ or Li^+ ions into the solution restores the prolonged electrical activity of the ureter. Under these conditions, the decrease of intracellular Ca^{2+} within 5 min was more than two times larger as compared with that in sodium-free medium. Upon substituting Ba^{2+} ions for Ca^{2+} ions in Krebs solution AP are generated within an interval which was the longer the higher the Ba^{2+} concentration in the medium. Li^+ ions can replace Na^+ ions in maintaining AP and in extruding calcium from the cell. It is supposed that the generation of the stable spike activity of the ureter depends on the functioning of Na^+—Ca^{2+}-exchange mechanism.

Key words: Ureter — Spontaneous activity — Intracellular concentration of Ca^{2+} — Exchange mechanism — Na^+-K^+-pump

Introduction

It is well known that the high electrochemical Ca^{2+} gradient across smooth muscle cell membrane maintains the spike activity (Kobayashi 1965; Shuba 1981). It is accepted that Na^+ ions can be responsible for the excitability threshold for Ca^{2+}. Thus, the basic depolarization for the calcium system in the ureter originates in the sodium plateau (Bury and Shuba 1976; Aaronson and

Benham 1989). However, in cells of the gasrointestinal tract, sodium-free medium can change the membrane polarization to the level of calcium channel activation (Kobayashi 1965; Sakamoto and Tomita 1982).

Ca^{2+} can be extruded from the cytoplasm by two transport systems: the ATP-dependent Ca^{2+} pump and the Na^{+}—Ca^{2+} exchange mechanism, which uses the energy of the sodium-gradient across the membrane (Eisner and Lederer 1985; Wuytack et al. 1985; Ashida and Blaustein 1987).

The presence of an Na^{+}—Ca^{2+} exchange system in the membranes of ureter smooth muscle cells has been confirmed (Aickin et al. 1984; Aickin 1987; Aaronson and Benham 1989). At the same time, investigations on isolated ureter cells of guinea-pig in normal physiological conditions showed the negligible role of exchange in Ca^{2+} extrusion from the cells (Aaronson and Benham 1989). However, a significant participation of Na^{+} has been suggested in the efflux of Ca^{2+} from the cytoplasm of cells with high levels of intracellular Ca^{2+}, such as aorta and arteries (Ashida and Blaustein 1987; Smith et al. 1989).

In the present paper an attempt has been made to show the role of Na^{+} in restoring the intracellular content of Ca^{2+} and, at the same time, in regulating the ureter spike activity; this is an indirect indicator of the involvement of the Na^{+}—Ca^{2+} exchange system in the above interrelated processes.

Materials and Methods

The experiments were carried out on isolated guinea-pig ureter dissected together with the renal pelvis from the perinephric region. After the isolation, the preparations were kept for an hour in Krebs solution at 36—37 °C, then put into testing solutions or mounted into the corresponding single "sucrose gap" chambers constructed according to Berger (1969) A constant velocity flow was maintained through all the chambers.

The Krebs solution had the following composition (in mmol/l): Na Cl 120.4; KCl 5.9; $CaCl_2$ 2.5; $NaHCO_3$ 15.5; NaH_2PO_4 1.2; $MgCl_2$ 1.2; glucose 11.5.

The sucrose solution prepared with tridistilled water, as well as the solution of potassium chloride were isotonic to the Krebs solution. The sodium-free isotonic solutions with increased concentration of Ca^{2+} ions were prepared from Krebs solution. The pH of the solutions was adjusted to 7.4. NaCl was replaced with an equivalent quantity of sucrose. The concentration of Ca^{2+} ions was changed from 2.5 to 15 mmol/l, with a corresponding decrease of the sucrose concentrations. In the lithium solution, NaCl was replaced by LiCl. Ouabain (10^{-4} mol/l) was added directly into the corresponding solutions. The role of the Na^{+}—K^{+}-pump in the generation of spontaneous activity was studied on muscles previously incubated with ouabain for 10 min. All the testing solutions were kept at 36 °C.

The intracellular $^{45}Ca^{2+}$ was estimated by the lanthanum method (Van Breemen et al. 1973). The muscles were previously kept for 2—3 hours in Krebs solution containing 1.64 μCi/ml $^{45}CaCl_2$ (Izotop, USSR) of a specific radioactivity of $3.78 \cdot 10^{2}$ Ci/mol (control). To enrich the intracellular content of calcium ions, the preparations were transferred to the corresponding sodium-free solutions containing $^{45}Ca^{2+}$. Then the muscles were immersed into the corresponding experimental

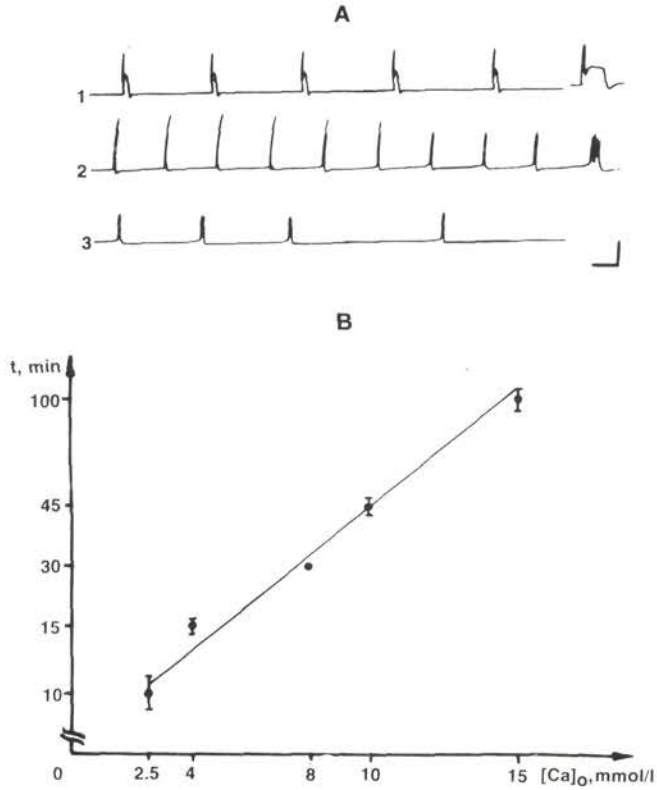

Fig. 1. Spontaneous activity of the guinea-pig ureter in sodium-free solution (*A*) and the dependence of the activity durationon on $[Ca^{2+}]_o$ (*B*). *A*. 1. Krebs solution. 2. Effect of the sodium-free solution at 4 mmol/l $[Ca^{2+}]_o$. 3. Inhibition of the activity in the sodium-free solution. Calibration: 20 mV, 4 s. To the right: 20 mV, 1 s. *B*. Ordinata: logarithmic scale. Abscissa: $[Ca^{2+}]_o$ in mmol/l. Each symbol represents the mean of three experiments ± S.E.M., except when smaller than the symbol size.

solutions, containing again $^{45}CaCl_2$. At the end of the experiment the muscles were kept in the lanthanum solution for 5 min; subsequently they were transferred to calcium-free solution containing 10 mmol/l $LaCl_3$. The study of $^{45}Ca^{2+}$ efflux in these conditions showed that $^{45}Ca^{2+}$ was completely washed out from the intracellular space within 50 min. Then, the muscles were dried on filter paper and weighed. The quantity of $^{45}Ca^{2+}$ was estimated after preliminary solubilization of the muscles, on an SL-4221 scintillation spectrometer (Roche-Bioelectronique Kontron, France) in Bray's scintillator (Bray 1960).

The records of experiments shown are typical results of 8—10 tests (electrophysiological investigations) or 3—4 series (radioisotope measurements).

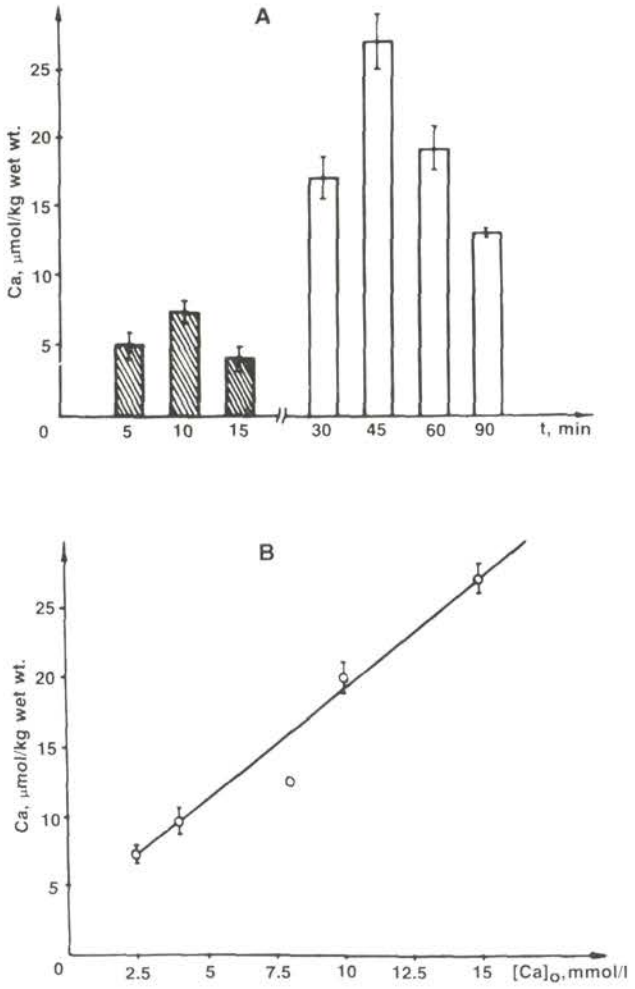

Fig. 2. Histograms of the intracellular Ca^{2+} content in dependence on the incubation time in sodium-free medium (*A*), and the dependence of the intracellular Ca^{2+} content on $[Ca^{2+}]_o$ (*B*). Hatched columns: $[Ca^{2+}]_o = 2.5$ mmol l; empty columns: 10 mmol/l. Each bar represents mean ± S.E. of three experiments.

Results

As shown in earlier investigations of cat ureter, spontaneous activity continued also in sodium-free medium, but the Ca^{2+} content had to be brought to 5 mmol/l (Kobayashi 1965). In our experiments spike activity of the guinea-pig ureter was

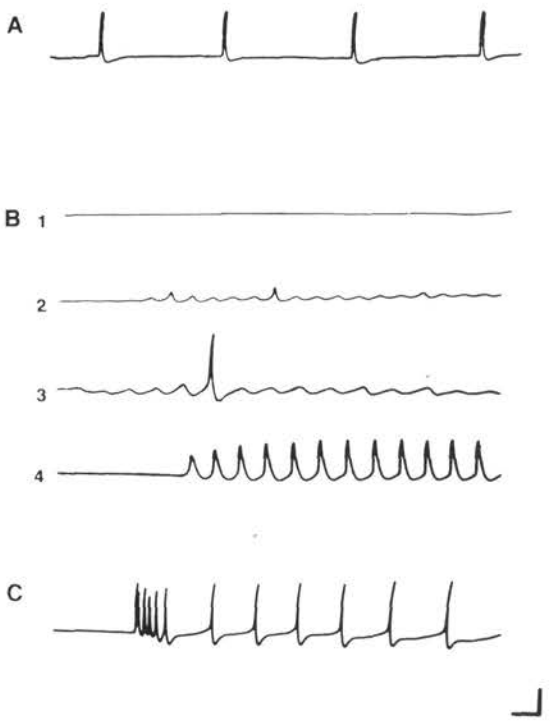

Fig. 3. Recovery of the spontaneous activity of the ureter incubated in sodium-free medium. *A*. Sodium medium (20 mmol/l Na^+ in sodium-free solution); *B*. In the presence of ouabain in sodium medium (10^{-4} mol/l), different $[K^+]_o$ and $[Na^+]_o$: 1. 126 mmol/l $[Na^+]_o$ and 5.9 mmol/l $[K^+]_o$; 2. 126 mmol/l $[Na^+]_o$ and 0 $[K^+]_o$; 3. 60 mmol/l $[Na^+]_o$ and 5.9 mmol/l $[K^+]_o$; 4. 60 mmol/l $[Na^+]_o$ and 3 mmol/l $[K^+]_o$. *C*. Sodium-free medium, 4 mmol/l $[K^+]_o$. The activity was restored within 2—3 min solution. Calibration: 10 mV, 4 s.

observed already at 2.5 mmol/l Ca^{2+} (Fig. 1*A*). This activity was recorded under these conditions also in preparations with no initial spontaneous rhythm. Noteworthy is the configuration of AP. As reported by several authors (Bury and Shuba 1976; Shuba 1977), the so-called Na^+ plateau disappears in sodium-free medium.

The results of the experiments showed that the logarithm of the activity interval length is linear by dependent on the Ca^{2+} content in the sodium-free medium (Fig. 1*B*).

Under these conditions it is natural to expect an increase in the intracellular content of Ca^{2+} to occur. The histogram in Fig. 2*A* indeed shows that the content of intracellular Ca^{2+} in the ureter tissue increases. In muscles incubated

in the sodium-free medium with a defined $[Ca^{2+}]_o$, the content of Ca^{2+} reaches its maximum within the interval of spike generation. Any prolongation or shortening of this time interval results in a decrease of the Ca^{2+} content in the ureter preparations (Fig. 2*A*). The dependence of the obtained maximal value of the intracellular content of Ca^{2+} on $[Ca^{2+}]_o$ is a linear function (Fig. 2*B*). The increase of the intracellular Ca^{2+} content can result in a level shift of membrane potential (Shuba 1981; Mangel et al. 1982; Jmari et al. 1986), in subsequent inactivation of voltage dependent calcium spike channels, and in the abolition of the activity (Jmari et al. 1986). The mechanisms responsible for the further decrease of Ca^{2+}-loaded cells were studied by introducting Na^+ into the medium. As illustrated in Fig. 3*A*, the inhibited activity in sodium-free solution is easily restored by the addition of at least 20 mmol/l Na^+ into the solution. As a result, continuous spike activity (over 40—50 min) appeared almost immediately. The amplitude and the rhythmicity of AP were slightly smaller than those in Krebs solution. The Na^+ ion in the medium has been attributed a certain role in maintaining the transmembrane potential close to the critical firing level; this ion maintains the operation of the electrogenic Na^+—K^+ pump and, moreover, it is characterized by a great permeability for the above structures (Casteels 1981; Aickin 1987). In this case, the introduction of Na^+ into the medium may be one of the reasons of Ca^{2+} spike channel activation.

Therefore, in a subsequent series of experiments we tried to restore the activity of the ureter in the presence of high concentrations of ouabain (10^{-4} mol/l) which should bring about complete inhibition of Na^+—K^+ pump operation (Aickin 1987). The muscles were preliminarily incubated for 10 min in the sodium-free medium with ouabain. This is sufficient for the inhibitor to suppress the spontaneous activity of ureter in Krebs solution (Kazarian and Tirayan 1984).

Under these conditions, activity could appear only in the presence of Na^+ and K^+ in the medium, and spikes were recorded for no longer than 15 min (Fig. 3*B*). A rather quick change of Na^+ intracellular activity has been shown for ureter cells upon changing the Na^+ content in the media in the presence of ouabain (Aickin 1987). Concerning our experiments it can be assumed that with muscles, previously incubated in a sodium-free solution, the indicated time interval (15 min) was sufficient for the intracellular Na^+ content to increase and for the transmembrane gradient to decrease. This may indirectly indicate the participation of Na^+—Ca^{2+} exchange in removing the intracellular Ca^{2+}.

Activity restoration (short, not exceeding 2 min) was observed also in the sodium-free medium. For this purpose solutions with a reduced K^+ content were used, and 50% of the preparations showed activity at 4 mmol/l K^+ (Fig. 3*C*).

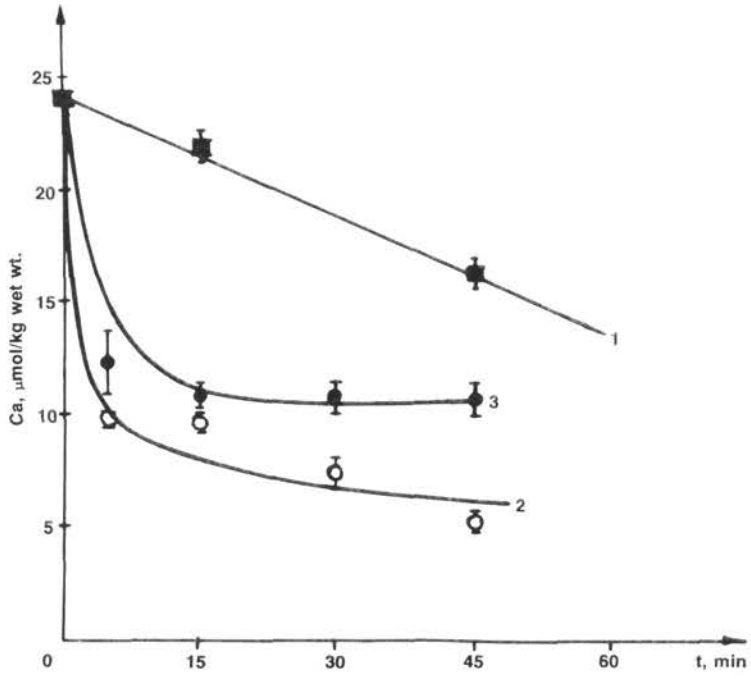

Fig. 4. The effects of Na^+ and Li^+ ions present in the external medium on intracellular Ca^{2+} content. The preparations were previously incubated for 45 min. in sodium-free medium at 10 mmol/l Ca^{2+} ions. (Zero point on the abscissa). 1. Sodium-free medium (control); 2. 120 mmol/l Na^+; 3. 120 mmol/l Li^+.

Thus, the presence of Na^+ in the medium is required for a prolonged generation of ureter spike activity to be restored.

It has been shown earlier for guinea-pig taenia coli (Ma and Bose 1977; Hirata et al. 1981) and for arterial preparations (Reuter et al. 1973) that a considerable fraction of Ca^{2+} efflux is activated by Na^+ present in the external medium.

Fig. 4 shows that in sodium containing medium the Ca^{2+} efflux from the cells is rather rapid, and substantially exceeds the monotonous decrease of intracellular Ca^{2+} observed in the sodium-free solution, as shown for cardiac muscle vesicles (Pitts 1979). For guinea-pig taenia coli, the efflux of Ca^{2+} activated either by Ca^{2+} or by Na^+ present in the external medium is sufficiently inhibited in the presence of Na^+ or Ca^{2+} respectively (Hirata et al. 1981). In this case, due to the presence of Ca^{2+} in the sodium containing medium, the Na^+-activated net efflux of Ca^{2+} can actually be slightly higher. Meanwhile, a high Ca^{2+} content in the sodium-free medium can activate the efflux of Ca^{2+} into the

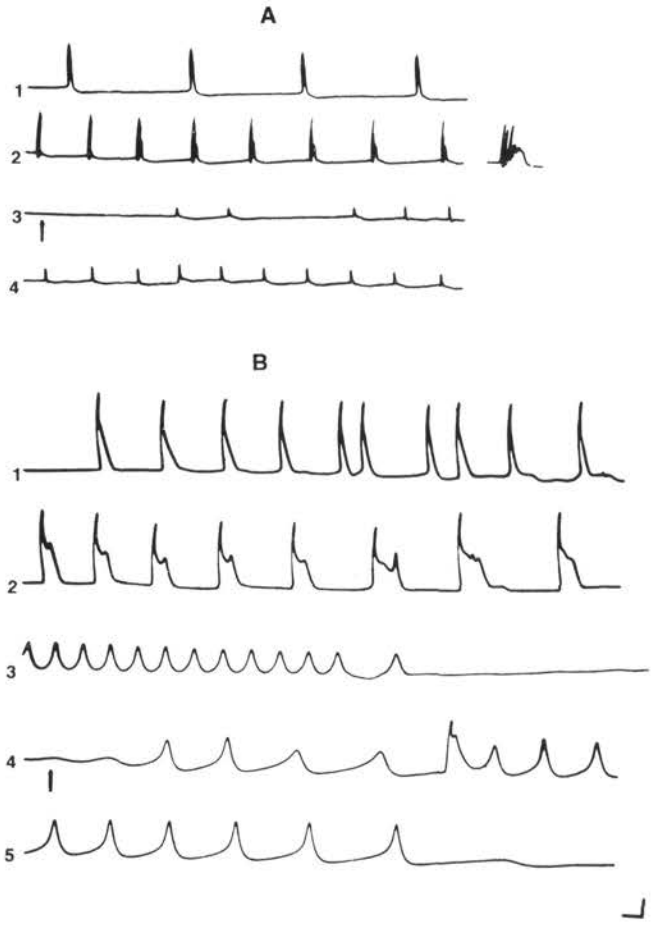

Fig. 5. The effects of Li^+ (*A*) and Ba^{2+} (*B*) ions present in the external medium on the spontaneous activity of the ureter muscle. *A*.1. Krebs solution. 2. Sodium-free solution. 3, 4. 20 mmol/l Li^+ ions added (arrow points to the beginning of the effect). *B*.1. Krebs solution. 2. Barium solution (2.5 mmol/l). 3. Suppression of activity in barium solution. 4. Ba^{2+} 25 mmol/l (arrow). 5. Suppression of activity. Calibration: 10 mV, 4 s. To the right: 10 mV, 1 s.

solution and thus promote the decrease of the intracellular content of that ion the way it is in the cardiac muscle (Busselen and Van Kerkhove 1978), barnacle muscles (Lederer and Nelson 1983; Rasgado-Flores and Blaustein 1987) and squid giant axon (Baker and McNaughton 1976). Nevertheless, the above experiments revealed that a considerable fraction of Ca^{2+} efflux is activated by Na^+ present in the medium.

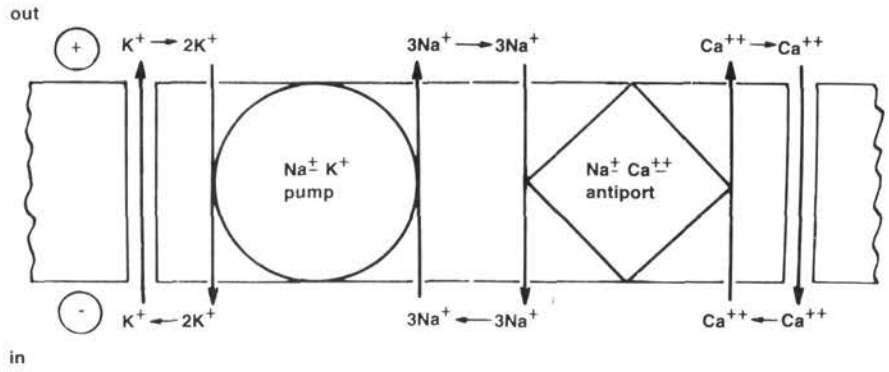

Fig. 6. Model of interaction of ion transporting membrane systems in ureter smooth muscle cells. The stoichiometry of the electrogenic Na^+ — Ca^{2+} exchange mechanism and the Na^+ — K^+ pump is taken conventionally to provide the cycles of Na^+, K^+ and Ca^{2+} ions between the cell and the medium.

The replacement of Na^+ by Li^+ in the external solution also revealed a considerable decrease of Ca^{2+} in ureter muscles incubated preliminarily in a sodium-free solution. At the same time, as shown in Fig. 4, the role of Li^+ in extruding Ca^{2+} is less obvious as compared with Na^+. In addition, the electrophysiological investigations showed that upon the introduction into the medium of Li^+, spikes appeared almost immediately, though with a smaller amplitude than with Na^+ (Fig. 5*A*). Thus, contrary to the squid giant axon in the ureter smooth muscle cells, Li^+ can replace Na^+ in the conjugate exchange with Ca^{2+}.

On the other hand, if Na^+ is a non-specific ion in the conjugate movement of Ca^{2+} from the cell within the Na^+—Ca^{2+} exchange mechanism, then the question arises concerning the specificity of this system to Ca^{2+}. The ion-exchanging Na^+—K^+ pump shows a high specificity to intracellular Na^+, i.e. to the ion which must be expelled from the cell. At the same time, there is no strict selectivity to K^+ which can be substituted by Rb^+, Cs^+ or NH_4^+ (Martirosov and Mikaelian 1970). If in Krebs solution the ureter can generate spikes spontaneously for a prolonged period of time, then upon replacing Ca^{2+} by Ba^{2+}, APs are recorded for a shorter period of time (Fig. 5*B*). The configuration of the Ba^{2+} spikes is considerably different from that of calcium AP. The slow component of AP was often widened and doubled (Fig. 5*B*, 2). Upon raising the Ba^{2+} content in the medium to 7 and 25 mmol/l, the disappeared spikes reapear for a short period of time (Fig. 5*B*, 4). The spikes are continuous in Krebs solution containing Ca^{2+}, whereas in barium medium with the same Na^+

content, AP are suppressed after a certain time, i.e. Na^+ cannot maintain the barium spike activity of the ureter smooth muscle cells.

Thus it can be assumed that the $Na^+ - Ca^{2+}$ exchange mechanism is involved in the regulation of spike activity of the ureter muscle. It is highly selective for Ca^{2+} and non-specific for Na^+.

Discussion

It has been shown for ureter, aorta and cardiac muscles (Kimura et al. 1987; Aaronson and Benham 1989; Smith et al. 1989) that under normal conditions of Ca^{2+} expelling from the cell (forward mode) the exchange system can be either of little effectiveness or simply inactive. The major role here is played perhaps by the low concentration of Ca^{2+} in the cell. An increase of the intracellular Ca^{2+} content *vice versa* will promote the activity of the antiporter (Ashida and Blaustein 1987; Smith et al. 1989). Actually, as appears from the results of the present investigations, an almost tenfold increase of the intracellular Ca^{2+} content induces a sharp efflux of Ca^{2+} as compared with the monotonous decrease of cell Ca^{2+} in sodium-free medium. During the first 5—8 min a similar quick efflux of Ca^{2+} is observed with cardiac sarcolemmal vesicles (Pitts 1979). The transport has been shown to proceed via the $Na^+ - Ca^{2+}$ exchange mechanism.

Curve 1 of Fig. 4 suggests the presence of other Ca^{2+}-expelling mechanisms. However, under the conditions of the present experiment it is Na^+ that plays a major role in Ca^{2+} expelling from the cell.

The Na^+-dependent decrease of intracellular Ca^{2+} is accompanied by a restoration of the spike activity upon introducing Na^+ into the medium (Fig. 3*A*). A similar picture was obtained with lithium solution, though with a smaller amplitude (Fig. 5*A*). The electrophysiological facts can be used to indirectly explain the maintenance of ureter activity by the operation of $Na^+ - Ca^{2+}$ antiporter.

The restoration of the spike activity observed in the presence of Na^+ (Fig. 3*A*) can be explained by triggerng electrogenic Na^+-transport systems which simultaneously maintain the excitability threshold of the ureter smooth muscle cell membranes and regulate the intracellular Na^+ and Ca^{2+} contents.

It was shown earlier that the spike activity of the ureter is coupled with the operation of the $Na^+ - K^+$ pump in such a way that switching out the pump results in a complete suppression of the activity (Kazarian and Tyrayan 1984). Since in the ureter the voltage-dependent channel generating the spike activity is the calcium channel, there must be some intermediate mechanism which couples $Na^+ - K^+$ pump with the operation of the Ca^{2+}-channel. In our opi-

nion, this mechanism is the electrogenic Na^+—Ca^{2+} exchange mechanism which controls the Ca^{2+} content in the cell. An interaction scheme of the ureter cell membrane ion mechanisms has been derived based on the possible relations between the above transport systems (Kazarian et al. 1989), the mechanisms are in full stoichiometric agreement (Fig. 6). As it has already been noted, in normal physiological conditions, Ca^{2+} which steadily enters the cell must be expelled by means of some other mechanisms, e.g. Ca^{2+}-pump (Aickin 1987; Aaronson and Benham 1989). Obviously a more complicated interaction scheme of membrane systems for Ca^{2+}, cannot be excluded. In this case, self-regulation of Na^+—Ca^{2+} antiporter is possible: at very low Ca^{2+} levels in the cell the Ca^{2+}-pump can regulate the Ca^{2+} content (Rasgado-Flores and Blaustein 1987). Nevertheless, indirect electrophysiological data support our ideas at least within the experimental conditions used in the present work.

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