Na^+–Ca^{++} Exchange Mechanism in Smooth Muscle of the Ureter

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Abstract. The participation of Na^+ in regulation of intracellular Ca^{++} content and in formation of spontaneous action potentials of guinea-pig ureter was studied. It was shown that the fast decrease of intracellular Ca^{++} in the Ca^{++}-loaded muscles was accompanied by enhancement of Na^+ content in the cells. The concentration gradient of Na^+ was found to define the effectiveness of Ca^{++}-extrusion from Ca^{++}-loaded cells. The decrease of intracellular Ca^{++} showed a sigmoidal dependence on Na^+ content in the medium.

A correlation was established between the concentration gradient of Na^+ and the formation of action potential plateau of ureter smooth muscle cells. The duration of action potential plateau decreased in accordance with Ca^{++} efflux and Na^+ influx. The results confirmed the participation of Na^+-Ca^{++} exchange mechanism in support of Ca^{++} cellular homeostasis as well as in the generation of action potentials of guinea-pig ureter.

Key words: Ureter — Spontaneous activity — Intracellular ion content — Exchange mechanism — Plateau phase

Introduction

Experimental investigations have shown the presence of Na^+-Ca^{++} exchange mechanism in the plasma membranes of ureter smooth muscle cells (Aickin 1987; Aaronson and Benham 1989). It is well known that Ca^{++} transmembrane translocation can be produced by this mechanism under the conditions of Na^+ concentration gradient (Mullins 1979; Ashida and Blaustein 1987; Rasgado-Flores and Blaustein 1987). The investigation of the exchange mechanism is complicated also by the existence of other transmembrane calcium flux supporting systems.

In accordance with data of Aickin (Aickin et al. 1984; Aickin 1987), when the Na^+-K^+ pump is inactivated, the Na^+-Ca^{++} exchange system directly participates in countergradiented concentration changes of Na^+ in ureter cells. However, investigations of arterial muscles and taenia coli cells confirmed the regulation of

intracellular Ca^{++} concentration by exchange mechanism under conditions of Ca^{++} overbase level (Katase and Tomita 1972; Ashida and Blaustein 1987; Smith and Smith 1987).

A similar role of the Na^{+}-Ca^{++} exchange mechanism was indirectly pointed out also in electrophysiological experiments in the ureter cells (Kazarian et al. 1991). However, for the further confirmation of participation of the exchange mechanism in maintaining Ca^{++} cellular homeostasis of the ureter it is necessary to reveal coincident Ca^{++} efflux and Na^{+} influx.

The problems concerning the regulation of spontaneous action potentials of ureter smooth muscle cells by Na^{+}-Ca^{++} exchange mechanism remains unsolved.

It is known that the complex action potential of ureter smooth muscle cells represents fast impulses of inward calcium current which are imposed onto the slow inward Na^{+} current (Bury and Shuba 1976; Shuba 1977). In this case we should consider the interconnection between the Na^{+} and Ca^{++} transfer systems. Actually, the increase of Ca^{++} concentration can be involved in the generation of slow inward current, forming plateau which is sensitive to Na^{+}-concentration gradient (Mitchell et al. 1984). The similar pattern is compatible with the operation of electrogenic Na^{+}-Ca^{++} exchange mechanism (Eisner and Lederer 1985; Ashida and Blaustein 1987; Aaronson and Benham 1989).

The aim of the present study was to attempt to estimate the role of the Na^{+}-Ca^{++} exchange mechanism in regulating the calcium cellular homeostasis and spontaneous development of action potentials in guinea-pig ureter.

Materials and Methods

The experiments were carried out on isolated guinea-pig ureters dissected together with the renal pelvis from the perinephric region. Each ureter was cut into three to four pieces, 1.2 to 1.5 cm long and about 500–600 μm wide. Electrical activity was recorded by means of the sucrose-gap method (Bulbring and Tomita 1969). After isolation the preparations were kept for an hour in Krebs solution at 36–37 °C and mounted into the corresponding sucrose-gap chambers. A constant velocity flow was maintained through all the chambers.

The Krebs solution had the following composition (in mmol/l): NaCl – 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 tridistillated 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^{++} were prepared from Krebs solution. The pH of the solutions was adjusted to 7.4. NaCl was replaced by an equivalent quantity of sucrose. Ouabain (10^{-4} mol/l) was added directly into the corresponding solutions. All the test solutions were kept at 36 °C.

Determination of calcium content in muscles

The intracellular $^{45}Ca^{++}$ 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, the Soviet Union), specific activity 3.78×10^2 Ci/mol (control).

To enrich the intracellular content of calcium ions the preparations were transferred to the corresponding sodium-free solutions containing $^{45}Ca^{++}$ Then the muscles were immersed into the corresponding experimental 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 a calcium-free solution containing 10 mmol/l $LaCl_3$ Under these conditions Ca^{++} efflux reached whole wash-off of intracellular space from Ca^{++} during 50 min while the membrane blocked by lanthanum preserved efflux of almost all cellular Ca^{++} Then the muscles were dried on filter paper and weighed The quantity of $^{45}Ca^{++}$ was estimated after preliminary solubilization of the muscles using an SL-4221 scintillation spectrometer (Roche Bioelectronique Kontron France) in Bray's scintillator (Bray 1960) The value of background cellular Ca^{++} (that is the intracellular concentration of Ca^{++} in Krebs solution) was taken into consideration which was subtracted from the intracellular concentrations of Ca^{++} in the corresponding solutions

Determination of sodium and potassium contents in muscles

The intracellular Na^+ and K^+ was estimated by a flame photometry method (El-Sharkawy and Daniel 1975) Fresh muscle strips were weighed and dried in an oven at 105°C for 48 h and reweighed To each muscle sample 3 ml of concentrated sulphuric acid and 0 05 ml of 33% hydrogen peroxide solution were added and the samples were dried at 200°C This treatment was repeated until a white residue was obtained This residue was then dissolved in 25 ml of double distilled water and the ion content was determined on a PMF flame photometer (the Soviet Union) The intracellular concentrations of sodium and potassium ions were calculated using calibration curves

A unit of 'absolute changes of content' was introduced in order to make the comparison of intracellular concentrations of Ca^{++} and Na^+ possible these being determined correspondingly in μmol/kg wet weight and mg/l This new unit is a dimensionless magnitude and is determined by the ratio of intracellular concentration of Ca^{++} (or Na^+) in the corresponding solution to the concentration of Ca^{++} (or Na^+) in Krebs solution

Results

Role of Na^+-Ca^{++} exchange in regulation of intracellular Ca^{++}

It is known that the pacemaker zone of ureter smooth muscle has the ability to generate the spike activity in sodium-free medium (Kobayashi 1969, Kazarian et al 1989a,b, 1991) Under these conditions the spike activity was observed also in ureter muscular bands which did not possess initial spike activity (Fig 1) As it was already pointed out, muscles preliminarily preserved in sodium free medium had a high intracellular content of Ca^{++} Under these conditions the concentration of Ca^{++} increased almost 10 times and intracellular content of Na^+ decreased more than 2 times (Fig 2) The cardiac Purkinje fibres under similar conditions showed a decrease of intracellular activity of Na^+ down to 0 mmol/l (Sonn and Lee 1988) That is why the introduction in medium with 120 mmol/l Na^+ created significant gradient and promoted Ca^{++} efflux Actually, after 45 minutes a marked decrease (about 3- fold) of intracellular Ca^{++} was observed and, correspondingly, the Na^+

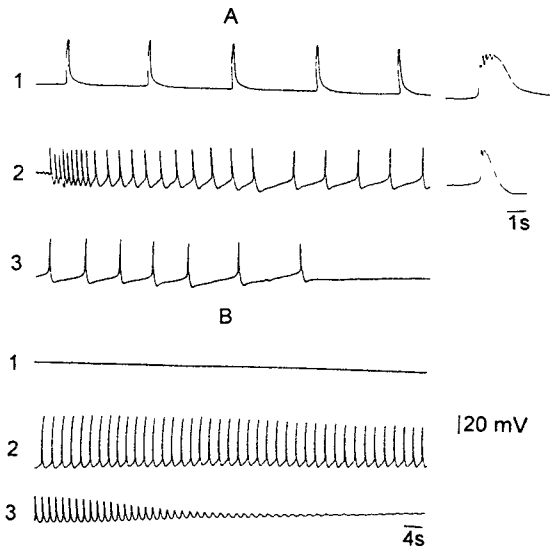

Figure 1. Spontaneous activity of the guinea-pig ureter in sodium-free medium. *A* – muscles with initial spike activity; *B* – muscles without initial spike activity. 1. Krebs solution. 2. Effect of the sodium-free solution (at 4 mmol/l $[Ca^{++}]_0$). 3. Inhibition of the activity in the sodium-free solution.

content increased more than 2- fold. Thus, the efflux of Na^+ per gradient was able to provide efflux of Ca^{++}.

It is known that the main role in the maintenance of transmembrane Na^+ concentration gradient is played by the electrogenic Na^+-K^+ pump. In this case the introduction of ouabain into the medium may eliminate the influence effects of Na^+ active transport on the value of Na^+-electrochemical gradient. Therefore, we accomplished a subsequent series of experimental investigations of Na^+-Ca^{++} exchange mechanism in the presence of ouabain in the medium.

The changes of Na^+ and Ca^{++} concentrations in the presence of ouabain apparently proved the inhibition of Na^+ active flux (Fig. 2). Meanwhile, introduction of ouabain in the sodium-free medium practically did not change the value of muscle Ca^{++} content. Simultaneously, in sodium medium this inhibitor slightly changed the intracellular Ca^{++} content of muscles.

Fig. 3 shows changes in time dependence of intracellular Ca^{++} and, correspondingly, increase of muscle Na^+ content in sodium containing medium (120 mmol/l). Na^+ inward and Ca^{++} outward fluxes via the Na^+-Ca^{++} exchange system is difficult to reveal on account of the existence ATP-dependent Ca^{++} mechanisms and passive diffusion. Therefore, attempt has been made to introduce corresponding correlations (Fig. 3). For Ca^{++} these correlations were made by transfer of Ca^{++} enriched muscles into the sodium-free solution (Fig. 3, curve 1). For Na^+ the corresponding correlation was estimated by transfer of the muscles into calcium-free solution while a normal Na^+ content was retained (120 mmol/l) (Fig. 3, curve 4).

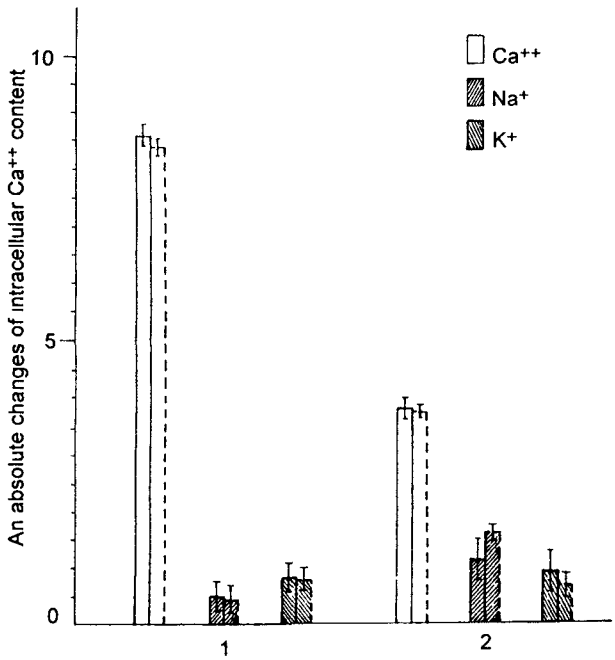

Figure 2. Histograms of absolute changes of the intracellular Ca^{++}, Na^+, K^+ content in dependence on the presence of $[Na^+]_0$ and ouabain (10^{-4} mol/l) in the external medium 1 Sodium-free solution (incubation time 45 min) 2 Next introduction into the sodium-free medium 120 mmol/l Na (incubation time 45 min) The data from ouabain containing medium is shown by stipple Each bar represents mean ± S E M of 6–8 preparations

Preliminary incubation of these muscles was carried out in sodium- and calcium-free solution

In sodium-free medium, calcium in muscles decreased more monotonously, while in the presence of 120 mmol/l Na^+ a fast Ca^{++} efflux took place for the first 10-15 minutes (Fig 3, curve 1), in agreement with recent studies (Pitts 1979; Reeves and Sutko 1979; Kazarian et al. 1991). After corresponding corrections, the curves of Na^+ inward and Ca^{++} outward fluxes were changed (Fig. 3, curves 2,3) These results can indirectly prove and help understand the various Ca^{++}-expelling processes, different from the Na^+-Ca^{++} exchange Nevertheless, the fast basic decrease of intracellular Ca^{++} during the initial period and a corresponding increase of Na^+ in cells indicate the prevailing role of Na^+-Ca^{++} exchange in the efflux of Ca^{++}, at least under these conditions (Fig 3).

If the Na^+-Ca^{++} exchange mechanism uses the energy of Na^+-electrochemical gradient for regulation of intracellular Ca^{++}, then the concentration of Na^+ in

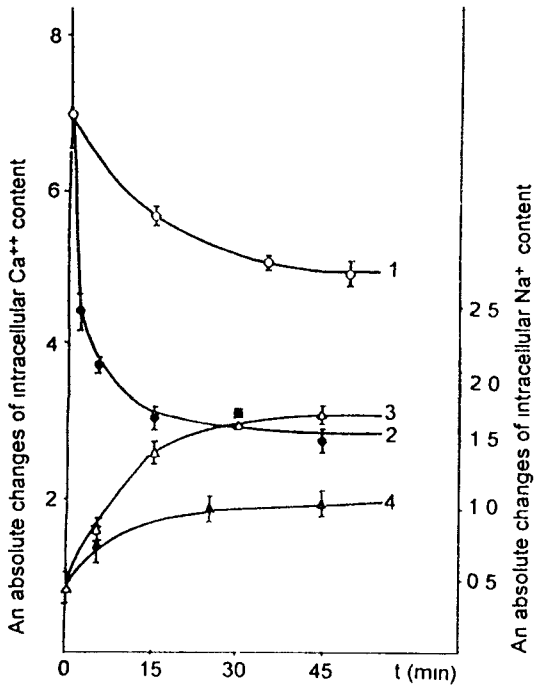

Figure 3. Absolute changes of the intracellular Ca^{++} (1, 2) and Na^{+} (3, 4) contents in dependence on time by adding Na^{+} ions in the medium 1 Sodium-free medium (○) 2,3 Sodium containing medium (120 mmol/l) (●, △) 4 Calcium-free medium (▲) All experimental solutions contained ouabain (10^{-4} mol/l) Each symbol represents mean ± S E M of 6–8 preparations

medium correspondingly affects the removal of cellular Ca^{++}. Fig. 4 shows the changes of intracellular Ca^{++} correspondingly to time for 3 different Na^{+} concentrations (0, 60 and 120 mmol/l) It is clear that after some time the concentration of intracellular Ca^{++} is independent of Na^{+} in medium (after 15 min curves 3 and 5 are almost parallel) Thus once more the participation of Na^{+}-Ca^{++} exchange mechanism in removing Ca^{++} is confirmed which takes place very effectively immediately after Na^{+} addition to the media (Pitts 1979; Reeves and Sutko 1979; Kazarian et al. 1989a)

At the same time the comparison of curves and results revealed that if during the introduction of 120 mmol/l Na^{+} in sodium-free solution, the Na^{+} dependent fraction of the Ca^{++} efflux from the cell was promptly activated (curve 5); on the other hand, this fraction of Ca^{++} efflux was reduced very slightly (curve 3), when half Na^{+} concentration from the external medium was removed Therefore, 60 mmol/l of Na^{+} in medium is enough for an effective Ca^{++} efflux.

The characteristics of the influence of Na^{+} electrochemical gradient on Ca^{++} efflux from the cell can be completely understood by investigation of the dependence of the intracellular Ca^{++} concentration on the presence of Na^{+} in medium (Fig 5). Calculation of the Ca^{++} content in muscles for each given Na^{+} concentra-

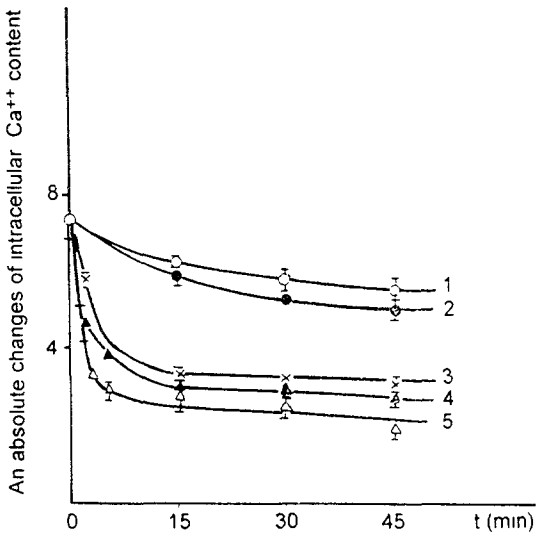

Figure 4. The change of intracellular Ca^{++} content in dependence on time (in the presence and absence of 10^{-4} mol/l ouabain) The preparations were previously incubated for 45 min in sodium-free medium at 10 mmol/l Ca^{++} (zero point on the abscissa) 1 Sodium-free medium without ouabain (○) 2 Sodium-free medium with ouabain (●) 3, 4, 5 Introduction of 60 mmol/l Na^+ and ouabain into the medium (×), 120 mmol/l Na^+ and ouabain (▲) 120 mmol/l Na^+ without ouabain (△) Each symbol represents mean ± S E M of 6-8 preparations

tion was done after 10 min The results show sigmoidal dependence which satisfies the condition of Ca^{++} expelling from the cell by Na^+ Ca^{++} exchange mechanism for cardiac muscles (Philipson and Nishimoto 1981, Kadoma et al 1982), cells of aorta (Smith et al 1989) and barnacle cells (Russel and Blaustein 1974) The curve approached the plateau when the concentration of Na^+ in the medium was higher than 80 mmol/l (Fig 5) At the same time, if maximal decrease in intracellular Ca^{++} was seen at high concentration of Na^+ in the medium (100 mmol/l), on the other hand half of this amount was removed already at 35–40 mmol/l Na^+ The measurement of Ca^{++} and Na^+ contents in muscles was accomplished simultaneously But despite the complex dependence of Ca^{++} changes on increase of Na^+ in the medium, intracellular Na^+ increase is monotonous (Fig 5)

From the linear dependence we can conclude that the increase of Na^+ in solution corresponds to a proportional increase of intracellular Na^+ concentration (at concentration less than 40 mmol/l, shifts from the linear dependence occur) Therefore, after 10 min the main efflux of Ca^{++}, at introduction of Na^+ into medium,

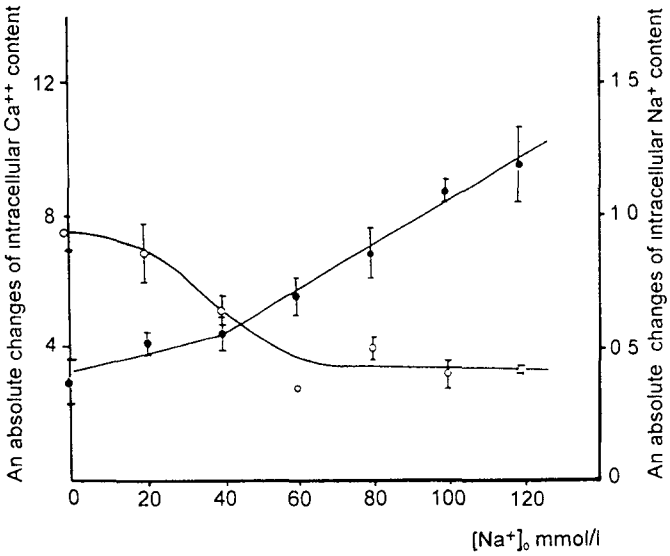

Figure 5. The effect of external Na^+ on intracellular Na^+ (●) and Ca^{++} (○) contents. Each symbol represents mean ± S.E.M. of 6–8 preparations.

stability was observed in the Na^+ concentration gradient for different concentrations of Na^+ in solutions (Fig. 5).

It can be concluded that the extrusion of Ca^{++} from the ureter cells is promoted by transmembrane gradient of Na^+ which determines the effectiveness of the Na^+-Ca^{++} exchange mechanism.

Participation of the Na^+-Ca^{++} exchange in formation of the action potential plateau

It has been shown that during generation of the action potentials of ureter the inward current is carried mainly by Ca^{++} (Kobayashi 1969; Bülbring and Tomita 1977; Shuba 1977). Na^+, which appears needful for the generation of stable rhythmic activity of the pacemaker zone of the ureter (Kazarian et al. 1989a, 1991), also takes part in the generation of the action potential (Kobayashi 1969; Bury and Shuba 1976). Fig. 6 shows the restoration of the activity of ureter muscles in sodium-containing medium (120 mmol/l), which were preincubated in sodium-free medium. At first the spikes had a considerably wide plateau (Fig. 6*A*, 1), which after 4–5 min decreased almost twofold. After this the duration of spike decreased almost to 1/4 of the initial size, meanwhile the amplitude slightly increased and a similar activity continued for a long time. For the muscles which did not restore spontaneous activity in sodium containing medium (in 40% cases), the action

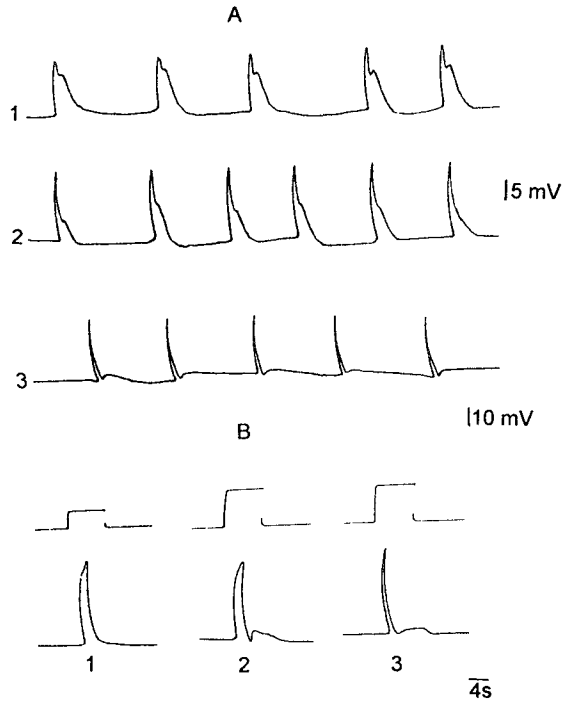

Figure 6. Recovery of activity of ureter smooth muscle cells by Na^+ (120 mmol/l) (*A*) and by electrical stimulation (*B*). *A*. 1, 2, 3. Immediately after replacement of the solution; after 4 min; after 7 min. *B*. 1, 2. Immediately after replacement of the solution; after 8 min. The force of stimulation was: 1 – 0.5 μA; 2 and 3 – 1 μA respectively. Top – intensity of current; bottom – membrane potential.

potentials evoked by depolarizing current of similar intensity immediately and 10 min after the replacement of sodium-free medium by a sodium-containing one (120 mmol/l), had different durations (Fig. 6*B*, 2 and 3). When the intensity of stimulation increased, the amplitude of the action potential decreased though its duration was almost stable (Fig. 6*B*, 1 and 2). According to these results one can see that the effect of Na^+ on the plateau of action potential was displayed mainly during the initial minutes after the introduction of sodium onto the medium.

As it was shown, the extrusion of Ca^{++} from the cell is quick enough (within the first 10 min) (Figs. 3 and 4). It is in complete accordance with the time interval during which the shortening of the action potential plateau was observed (Fig. 5).

Also the role of Na^+on the formation of plateau was investigated when the Na^+-pump was inhibited. As it is known, this is the mechanism of maintenance of the Na^+ concentration gradient. In ouabain containing medium under low Na^+

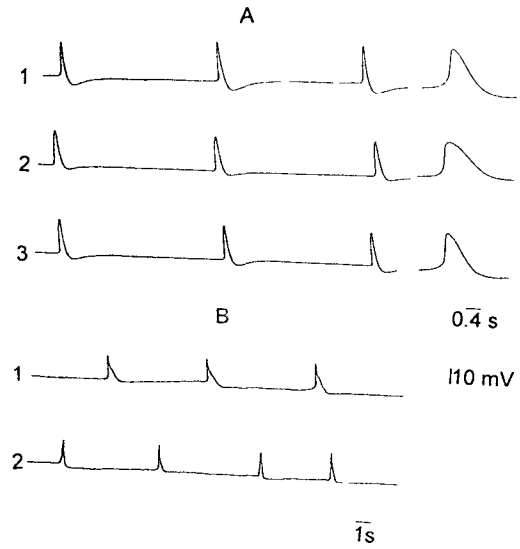

Figure 7. The effect of Na^+ concentration gradient on the recovery of activity in presence of ouabain (10^{-4} mol/l). *A*. 1 Concentration of Na^+ ions in medium was 30 mmol/l. 2 and 3 – After 4 min the concentration of Na^+ increased to 80 mmol/l; after 5 min. *B*. 1 and 2 – Concentration of Na^+ in medium was 100 mmol/l; after 10 min.

concentrations (30–40 mmol/l) the activity appeared as simple peak discharges, which were eliminated during 3–4 min (Fig. 7*A*, 1). The following increase of the Na^+ content in the medium (before 80–100 mmol/l) led to the restoration of spikes with longer duration. The duration of these spikes then is shortened and the generation time does not exceed 5–6 min (Fig. 7*A*, 2 and 3). In those cases when the sodium-free medium was replaced by a solution with high Na^+ concentration, a considerable plateau was reached and the activity was recorded during 15 min. As it was shown above (Fig. 3), under these conditions, during this interval we observed a 3-fold increase of Na^+ gradient and a more than 2-fold increase of Ca^{++}. The latter may be the cause of disappearance of plateau and activity (Kazarian et al. 1989a, 1991).

Thus a certain effect of Na^+ concentration gradient can be ascertained which provides the extrusion of excess Ca^{++} content from the cell for formation of action potential by the regulation of the duration of the plateau phase.

Discussion

In isolated smooth muscle cells of ureter some participation of the Na^+-Ca^{++} system was found in countergradiented transport of Ca^{++} from cells under normal

physiological conditions (Aaronson and Benham 1989). It had been revealed that the exchange of Ca^{++} efflux played a negligible role in the basic levels of Ca^{++}. Perhaps, due to the low concentrations of Ca^{++} in cells the exchange system is either ineffective or simply inactive similar to smooth muscles of aorta (Smith et al. 1989), cardiac muscles (Kimura et al. 1987) and squid giant axon (DiPolo and Beauge 1983). On the contrary, an increase of the intracellular Ca^{++} will promote the activity of the Na^+-Ca^{++} exchange mechanism.

These experiments revealed that for Ca^{++}-loaded muscles the exchange played a major role in the fast extrusion of Ca^{++} from the ureter cells. At the same time it was shown that the value of Na^+ concentration gradient determined the amount and the velocity of Ca^{++} extrusion from the cells (Figs. 4 and 5). At high concentrations of Na^+ in the medium this velocity reached a stable maximal value, which during further 10–15 min promoted a basic decrease of intracellular Ca^{++}.

Thus, during the increase of intracellular Ca^{++} a prevailing role in Ca^{++} extrusion from the cell belongs to the Na^+-Ca^{++} exchange mechanism. This does not depend on the effectiveness of the participation of the Na^+-Ca^{++} exchange mechanism in the regulation of the basic level of intracellular Ca^{++}.

At the same time it was known that the Na^+-Ca^{++} exchange mechanism operates immediately in generation of spontaneous action potentials of ureter (Kazarian et al. 1989b, 1991). In the absence of Na^+ in the medium a slow inward calcium current may take part in the formation of action potential plateau (Kochemasova 1971, 1982), but under normal conditions, in our point of view, this role belongs to exchange currents from the whole complex of simultaneous transmembrane currents. Actually, the presence of voltage dependent fast calcium channels in membrane (Kobayashi 1969; Bury and Shuba 1976) leads to the increase of intracellular Ca^{++} concentration and decrease of the reversal potential of exchange mechanism, respectively. This in turn promotes the conditions for the generation of inward currents by the Na^+-Ca^{++} system, which are sensitive to Na^+-concentration gradient (Figs. 6 and 7).

After shortening of the action potential plateau in ouabain containing solutions, the restored activity was eliminated unlike Krebs solution where the amplitude of action potentials had increased (Fig. 6). Perhaps, the extrusion of the intracellular Ca^{++} excess in such cases restored Ca^{++}- and Na^+- electrochemical gradients and decreased the electrogenic component of the Na^+-Ca^{++} exchange system. The latter, on the level of membrane polarization, influenced the restoration of the Na^+-K^+ pump and therefore, on involving the whole interconnected transport systems, which promoted the generation of normal action potentials (Kazarian et al. 1989a,b).

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