Effects of Temperature and Na_0^+ on the Relaxation of Phasic and Tonic Tension of Guinea-Pig Ureter Muscle

Th. V. BURDYGA[1] and I. S. MAGURA[2]

1 *Department of General Physiology, Institute of Physiology, Kiev State Shevchenko University, Kiev 17, 252017, USSR*

2 *Department of General Physiology of the Nervous System, Bogomoletz Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev 24, 252601 GSP, USSR*

Abstract. Effects of temperature and Na_0^+ on the relaxation of guinea-pig ureter smooth muscle were studied. Relaxation of phasic contraction was found to be highly temperature-dependent, practically independent of Na_0^+ and Ca_0^{2+}, and resistant to vanadate. The relaxation of the tonic tension of both high-K and low-Na contracture was less temperature-dependent and affected by Na_0^+. The relaxation of tonic tension produced by introduction of Na_0^+ was about 3—5 times faster than that produced by Ca-free solution. La^{3+} ions were found to block the relaxation of the tonic component of the Na^+-free contracture initiated by removal of Ca_0^{2+}. Three systems of regulation of cell calcium are suggested to be operative in the ureter muscle: a fast one which is highly temperature-dependent and responsible for the relaxation of the phasic contraction (probably the sarcoplasmic reticulum), and two slow membrane-linked carriers, one of which is dependent on Na_0^+ (probably Na—Ca exchange) and another one which is independent of Na_0^+ and inhibited by La^{3+} (probably Ca-pump).

Key words: Ureter muscle relaxation — Sarcoplasmic reticulum — Na—Ca exchange

Introduction

Results of physiological and pharmacological studies reported by a number of investigators suggest that the initial event in excitation-contraction coupling of the guinea-pig ureter muscle is an increase in the membrane permeability to external Ca^{2+} (Washizu 1967; Kuriyama and Tomita 1970; Shuba 1977; 1981; Brading et al. 1983). Under normal conditions the ureter muscle generates phasic contractions associated with the action potential. However, tonic tension

associated with sustained depolarization is normally seen when the ureter muscle is placed in high-K solution (Washizu 1967; Sunano 1976; Johnishi and Sunano 1978). Also, it has been shown recently that Na-loaded ureter maintained tonic tension associated with Ca influx on the mechanism of Na—Ca exchange (Aickin et al. 1984). Thus it has been suggested that Ca ions can enter the cell via both inactivating and noninactivating voltage-operated Ca channels and Na—Ca exchange (Kochemasova and Shuba 1979; Aickin et al. 1984). To bring about relaxation of a contracted muscle, the relaxing system must successfully compete with the contractile proteins for Ca ions and remove Ca^{2+} from the cytoplasm. The recovery of the internal Ca^{2+} level and the mechanism of relaxation of the ureter muscle has not been studied as yet.

A reduction of Ca_i^{2+} can be accomplished by intracellular Ca sequestering stores and the cell membrane linked Ca^{2+}-extrusion systems (van Breemen et al. 1973; Penniston 1983; Bond et al. 1984). The intracellular organelles usually involved in Ca^{2+} movements are the sarcoplasmic reticulum (SR) and mitochondria (Loutzenhiser et al. 1985; Jundt et al. 1975).

Obviously, both SR and mitochondria cannot serve as a longterm source or sink of large amounts of Ca^{2+}, because their storage capacity is limited although they might be important in the control of intracellular Ca^{2+} on a short-time scale (Penniston 1983; Bond et al. 1984). The physiological significance of SR is well documented (Somlyo et al. 1979; Somlyo 1984; Bond et al. 1984), while the mitochondrial role in the intracellular Ca^{2+} buffering has recently been questioned (Somlyo 1984).

The limited Ca^{2+}-storing capacity of the intracellular Ca buffering systems on one hand, and increased Ca permeability of the cell membrane during activity on the other hand, strongly suggest the involvement of the cell membrane in the regulation of Ca^{2+} movements, especially on a long-time scale.

The Na—Ca exchanger and the ATP-dependent Ca^{2+} pump are the two known ways by which cells export Ca^{2+} against steep concentration gradient between the cytoplasm and the extracellular space (Blaustein and Nelson 1982; Schatzman 1982; Casteels et al. 1985). The existence of the Na—Ca exchanger in the ureter muscle was suggested in previous study (Aickin et al. 1984). However, the role of the exchanger in relaxation of the ureter smooth muscle was not studied in this previous work.

In the present study the effects of temperature and Na_0^+ on the relaxation of phasic and tonic tension were studied.

Materials and Methods

Simultaneous electrical and mechanical records were obtained from isolated pieces of whole ureter from guinea-pig using the double sucrose gap method (Bülbring and Tomita 1969). Action poten-

tials were evoked by just suprathreshold depolarizing current pulses (in the order of 10^{-7} A) of short duration (20—50 ms) to avoid the possible influence of prolonged depolarization on the shape and amplitude of the action potential. Anelectrotonic potentials were evoked by rectangular current pulses of the order of 10^{-7} A and 2—3 s duration.

Tension alone was recorded with the continuous superfusion technique described in detail by Brading and Sneddon (1980). Four tissue pieces of about 1 cm length could be studied simultaneously by the use of four chambers, and contractions were recorded on a 12 channel loop oscilloscope.

The modified Krebs solution used in the majority of the experiments was of the following composition (mmol/l): Na^+ 120.3; K^+ 5.9; $Tris^+$ 16.6; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 150.2; glucose 11.5; equilibrated with 100% O_2, pH 7.4.

Drugs used were: procaine, lidocaine, tetraethylammonium chloride (TEA), ouabain (all B.D.H. U.K.) and nifedipine (BAY U.K.). High-K^+ solution was prepared either by replacement of all NaCl by KCl (isotonic solution) or by adding KCl as solid salt to Krebs or Na^+-free solution.

Loading of the tissue with Na^+ was done as described previously (Aickin et al. 1984).

Results

Influence of temperature on relaxation of twitch tension

Twitch tension which usually develops during the action potential normally commences upon repolarization of the action potential and decays in a rather simple manner. Fig. 1*A* shows typical phasic contractions associated with the

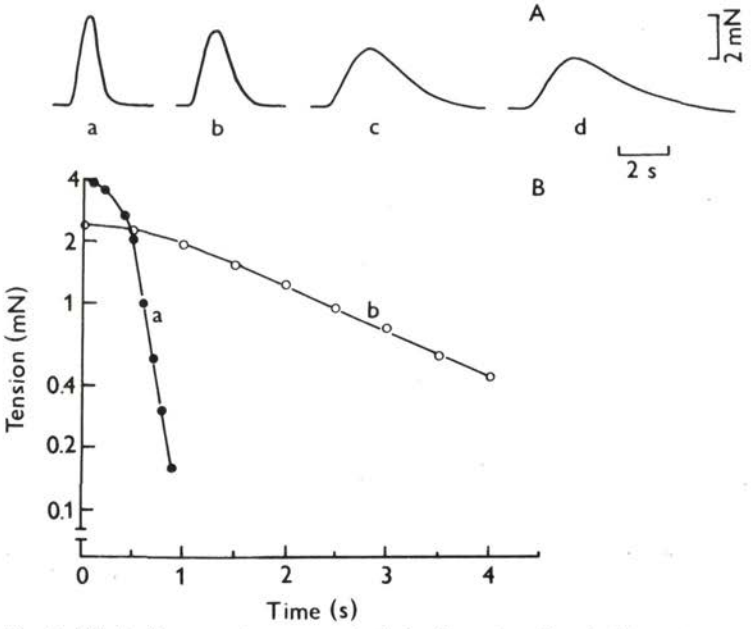

Fig. 1. Effect of temperature on evoked phasic contractions in the ureter smooth muscle. (Tension recording, field stimulation). *A*, *a—d*, recordings of the phasic contraction from the same preparation at 37°C, 33°C, 27°C and 22°C. *B*, semilogarithmic presentation of relaxation of the phasic contraction at 37°C (●) and 22°C (○). ○ — $\tau = 2200$ ms; ● — $\tau = 150$ ms.

evoked action potentials recorded at various temperatures. The semilogarithmic plots of the relaxation of the phasic contraction at 37°C and 22°C are shown in Fig. 1*B*; it can be seen that relaxation is mainly exponential with a time constant, τ, of 150 ms at 37°C (Fig. 1*B*,*a*). The time constants of the main part of the relaxation phase were scattered and values ranging between 150—250 ms were obtained. The average time constant was 190 $\pm$ 18 ms ($n = 6$). In accordance with observations in cardiac muscle (Goto et al. 1972) the time constant was shown to have a slight dependence on the amplitude of the twitch tension. Relaxation was somewhat slower when the tension developed was small.

Fig. 1*A* shows that lowering the temperature reduced both the amplitude and rate of rise and fall of the phasic contraction. The semilogarithmic plot of the relaxation phase of twitch tension at 22°C is shown in Fig. 1*B*,*b* giving the value of τ 2200 ms which is roughly 10 times higher than that measured at 37°C (Fig. 1*B*,*a*).

Twitch tension fall in Na-free solution

Complete removal of Na_0^+ normally abolishes the plateau component of action potential resulting in a reduction of phasic contraction amplitude (Fig. 2*B*). However, introduction of K^+-channel blockers (TEA, 5 mmol/l) or local anaes-

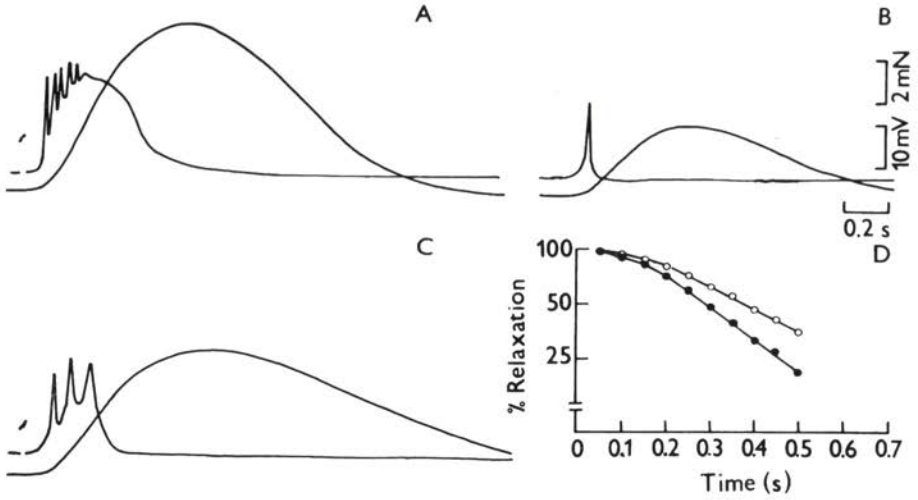

Fig. 2. Effect of Na^+-free solution on electrical and mechanical response of the ureter muscle (double sucrose-gap method). Evoked electrical (upper trace) and mechanical (lower trace) activity in Krebs solution (*A*), in Na^+-free (Tris substitution) solution (*B*) and in Na^+-free solution with 1 mmol/l QX-314 added (*C*). *D*, semilogarithmic plots of relaxation of phasic contraction in Krebs solution (●) and Na^+-free solution with QX-314 added (○). In this and subsequent Figures the semilogarithmic plots are represented as percentage of relaxation with the initial level of tension taken for 100%. ○ — $\tau = 350$ ms; ● — $\tau = 250$ ms.

thetics (procaine, lidocaine or QX-314, 1 mmol/l) induced a partial recovery of the amplitude of phasic contractions associated with a partial recovery of the plateau of action potential (Fig. 2*C*). All these agents initiated spontaneous mechanical activity superimposed on slowly rising tonic tension. Analysis of the time course of relaxation of the phasic contractions associated with action potential showed that the time constant was only little changed (Fig. 2*D*). The average time constant was 250 ± 25 ms ($n = 5$). Reintroduction of Na_0^+ immediately relaxed the tonic tension and had practically no effect on the time course of relaxation of the phasic contractions. As in Krebs solution, cooling of the ureter muscle in Na^+-free solution to 22°C caused 10—15-fold slowing of relaxation of the action potential mediated phasic contraction.

Effect of Ca^{2+} and vanadate

Variation of Ca_0^{2+} (0.1 25 mmol/l) had little effect on the time course of twitch relaxation of ureter muscle. Vanadate (10^{-4}—10^{-3} mol/l), a putative blocker of Ca pump of the cell membranes (Penniston 1983), initiated spontaneous activity and had no effect on the time course of relaxation of the phasic contraction.

Effect of nifedipine

Nifedipine (10^{-5} mol/l), a putative blocker of voltage-operated Ca channels, which was shown to cause a progressive fall in the amplitude of the twitch tension associated with the shortening of the plateau of the action potential (Brading et al. 1983) had no effect on the time course of relaxation of the phasic contractions.

Relaxation of tonic tension induced by high-K^+ solution

When ureter muscle was immersed in high-K^+ (126 mmol/l) solution (all NaCl replaced by KCl), depolarization of the membrane initiated at first several action potentials which produced phasic contractions followed by tonic tension associated with sustained depolarization (Fig. 3*A*). Since high-K^+ solution was also Na^+-free the tonic tension might have been partly potentiated due to the absence of Na_0^+. To study this, the effect of elevation of K_0^+ in Na^+-containing solution was studied by addition of KCl as solid salt to Krebs solution. To exclude possible effect of tonicity, the effect of hypertonic high-K^+ Na^+-free solutions containing Tris HCl, on ureter muscle was also studied. Typical experimental records of the resultant contractures are illustrated in Fig. 3*B*. Fig. 3*B*,*a* shows a typical response of the ureter muscle to isotonic high-K^+ (Na^+-free) solution. The contracture induced by hypertonic high-K^+ (100 mmol/l) solution, which was also Na^+-free, was basically the same as that induced by isotonic high-K^+ solution (Fig. 3*B*,*b*). However, elevation of K_0^+ in the presence of Na_0^+ produced tonic tension which was lower than that seen in Na^+-free

either isotonic or hypertonic solution (Fig. 3*B*,*c*). Removal of Na_0^+ in the course of the development of the tonic tension caused elevation of the latter (Fig. 3*B*,*d*). Nifedipine (10^{-5} mol/l) completely blocked the contractile response of the ureter muscle to both isotonic or hypertonic high-K^+ solution. When Ca_0^{2+} was removed in the course of the development of tonic tension induced by high-K^+ solution, a slow relaxation was normally seen. Effect of Na_0^+ and temperature on the relaxation of tonic tension produced by Ca removal was studied in subsequent experiments.

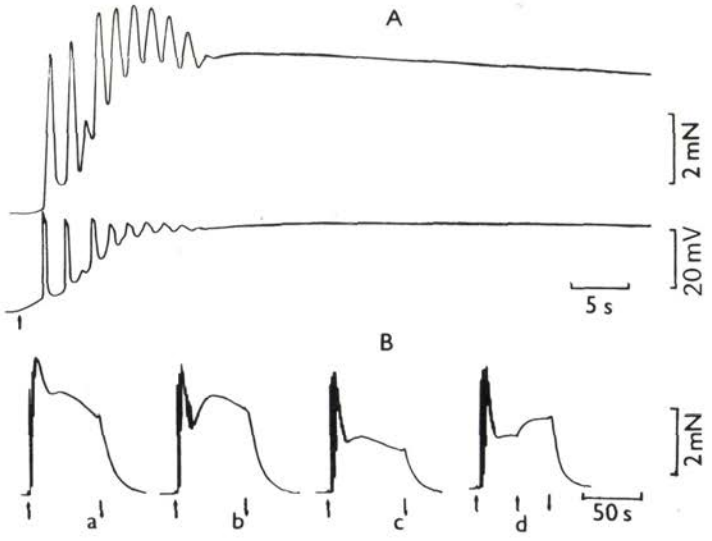

Fig. 3. Effect of high-K^+ solution on the ureter smooth muscle. *A*, electrical (lower trace) and mechanical (upper trace) response of the ureter muscle to isotonic 126 mmol/l—K^+ solution (double sucrose gap method). *B*, contractures of the ureter muscle induced by isotonic 126 mmol/l—K^+ (all NaCl replaced by KCl) solution (*a*); hypertonic 100 mmol/l—K^+ Na^+-free solution (all NaCl was replaced by $Tris^+$ and KCl was added as solid salt) (*b*); hypertonic 100 mmol/l—K^+ Krebs solution (KCl was added to Na^+-containing Krebs solution as solid salt) (*c*). *B*,*d* shows elevation of tonic tension by Na_0^+ removal (all NaCl was replaced by Tris HCl; the contracture was induced by hypertonic 100 mmol/l—K^+ Krebs solution). Note the depression of tonic tension in the presence of Na_0^+.

Effect of Na_0^+ and temperature on the relaxation of tonic tension

Fig. 4*A*,*B* shows typical records of the time course of relaxation of tonic tension of high-K^+ (126 mmol/l) contracture induced by Ca^{2+}-free solution (with 2 mmol/l EGTA) with or without Na_0^+ added both at 37°C and 22°C. The respective semilogarithmic plots of the relaxation illustrated in Fig. 4*C* yielded a time constant of 37 s in Na^+-free (*b*) and 13 s in Na^+-containing solution (*a*). Average time constants of relaxation of tonic tension in Na^+-free solution were

32 ± 7 s and 12 ± 5 s ($n = 5$) in the Na^+-containing solution. These results suggest that Na_0^+ has a strong accelerating effect on the relaxation of tonic tension of high-K^+ contracture. Cooling potentiated high-K^+ contracture (Fig. 4*B*), however, the time course of relaxation of tonic tension induced by Ca^{2+}-free solution in both Na^+-containing and Na^+-free solution was practically unaffected by lowering the temperature. The semilogarithmic plot of the relaxation produced by Ca^{2+}-free Na^+-containing solution at 22°C is shown in Fig. 4*C*. The time constant of the linear part of relaxation was basically the same. These results suggest that Na—Ca exchange is involved in relaxation of tonic tension of high-K^+ contracture.

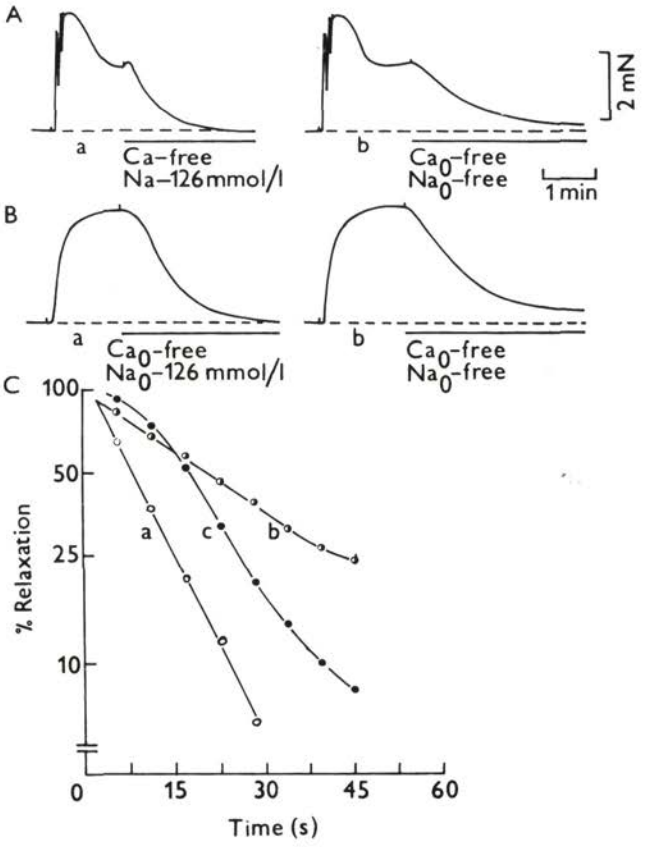

Fig. 4. Effect of Na_0^+ and temperature on the relaxation of tonic tension of high-K^+ contracture produced by Ca^{2+}-free solution with 2 mmol/l EGTA. The contracture was evoked by isotonic 126 mmol/l—K^+ solution (tension recording). *A*, the time course of recovery in Ca^{2+}-free solution with (*a*) and without (*b*) Na_0^+ added; *B*,*a*,*b* the respective records taken from the same preparation at 22°C. C, semilogarithmic plots of the relaxation of tonic tension with Na_0^+ added at 37°C (○) and 22°C (●) and without Na_0^+ at 37°C (◑). ○ — $\tau = 12.5$ s; ● — $\tau = 16$ s; ◑ — $\tau = 37$ s.

Relaxation of low-Na^+ contracture of Na^+-loaded ureter muscle

In previous study it was found that the ureter muscle exposed to ouabain or K^+-free solution gained restricted quantity of Na ions and responded with strong Ca_0^{2+}-dependent contracture upon removal of Na_0^+ associated with membrane hyperpolarization (Aickin et al. 1984). Studies of relaxation from such contractures in the ureter muscle have shown that relaxation of low-Na^+ contracture upon reapplication of Na_0^+ is rapid and is practically unaffected by temperature (in the range of 22°C—37°C) (Fig. 5). The semilogarithmic plots give the time constants of 13 s at 37°C (Fig. 5*D*,*a*) and 16 s at 22°C (Fig. 5*D*,*b*) implying that the major mechanism producing relaxation is Na—Ca exchange. Cooling significantly potentiated the low-Na^+ contracture and markedly slowed the relaxation of the 'phasic' component of the low-Na^+ contracture. The semilogarithmic plots of spontaneous relaxation of the 'phasic' component gave the time constants of 18 s at 37°C (Fig. 5*C*,*a*) and 80 s at 22°C (Fig. 5*C*,*b*). These results strongly suggest that some energy-dependent system of Ca^{2+} uptake

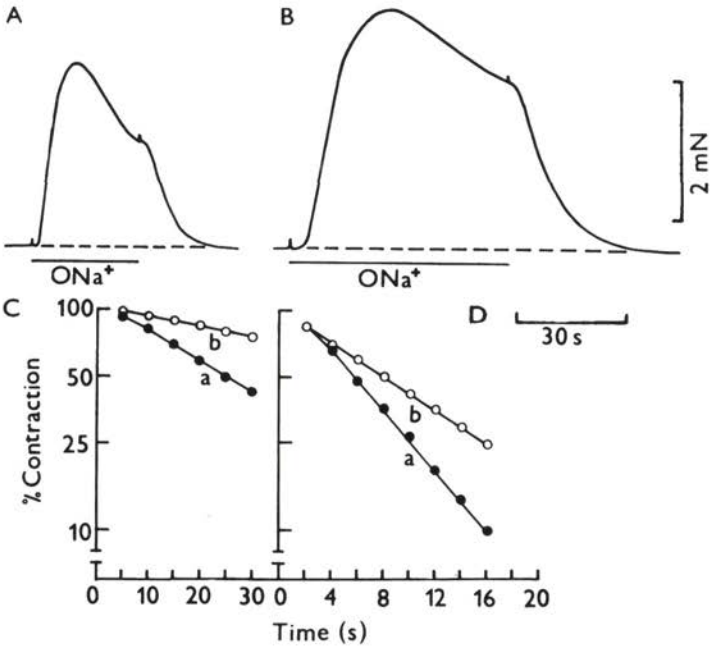

Fig. 5. Effect of temperature on Na^+-free contracture of Na^+-loaded ureter muscle. *A*,*B* low-Na contracture generated by Na^+-loaded ureter upon complete withdrawal of Na_0^+ ($Tris^+$ substitution) at 37°C and 22°C, respectively. *C*, semilogarithmic plots of spontaneous relaxation of low-Na^+ contracture at 37°C (●) and 22°C (○); *D*, semilogarithmic plots of relaxation of low-Na^+ contracture induced by reintroduction of Na_0^+ at 37°C (●) and 22°C (○).

operates in the ureter muscle and works against elevation of the intracellular concentration of Ca^{2+} entering the cell via Na—Ca exchange.

When Ca_0^{2+} was removed from the bathing fluid during the development of low-Na^+ contracture, relaxation was normally seen. However, this relaxation induced by Ca^{2+}-free solution was 3—5 times slower and less complete than that seen upon reintroduction of Na_0^+ (Fig. 6*A*,*B*). The semilogarithmic plot of the relaxation produced by Ca^{2+}-free solution gave the value of $\tau = 60$ s (Fig. 6*C*,*b*).

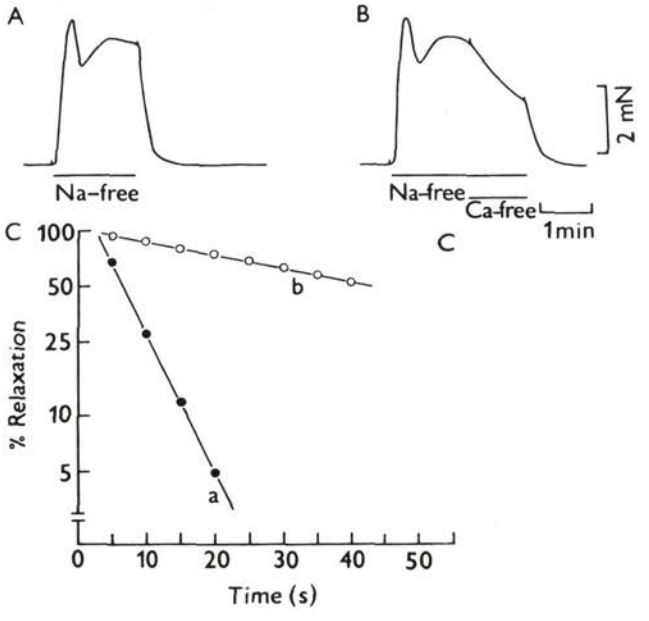

Fig. 6. Experimental records to compare the time course of relaxation of low-Na^+ contracture induced by readmission of Na_0^+ (*A*) and removal of Ca_0^{2+} (Ca^{2+}-free solution with 2 mmol/l EGTA) (*B*). *C*, semilogarithmic plots of relaxation of low-Na^+ contracture induced by readmission of Na_0^+ (●) and Ca^{2+}-free solution (○). Note that the relaxation produced by Ca^{2+}-free solution is much slower than that induced by reintroduction of Na_0^+. Relaxation produced by Ca^{2+}-free solution is immediately increased upon the addition of Na_0^+. ○ – $\tau = 60$ s; ● – $\tau = 9$ s.

Fig. 6*B* also shows that introduction of Na_0^+ in the course of relaxation produced by Ca^{2+}-free solution immediately increased the relaxation rate in spite of the presence of Ca_0^{2+} in the Na^+-containing solution. This quasi-instantaneous alteration in the relaxation rate induced by Na_0^+ suggests that Na ions act on some membrane mechanism which has external Na^+ sites. This implies that the mechanism that delivers Ca^{2+} and the one that pumps it out is the same and it reflects the operation of Na—Ca exchange. However, slow relaxation of

the tonic component of the low-Na^+ contracture produced by removal of Ca^{2+} from the bathing fluid suggests the existence of other systems of Ca removal from the cytoplasm which are likely to be membrane-linked Ca-pump or Ca-pump of the intracellular Ca stores.

Previously it was found that Ca-pump of the cell membrane could be selectively blocked by either vanadate (Penniston 1983) or La^{3+} (Sarkadi et al. 1977). Vanadate was found to potentiate the tonic tension of low-Na^+ contracture in concentration as high as 1 mmol/l but did not abolish the relaxation of the tonic tension induced by Ca^{2+}-free solution. On the other hand, this relaxation was completely blocked by La^{3+} at 5 mmol/l (Fig. 7*A*). In other words, La^{3+} in the absence of Na_0^+ and Ca_0^{2+} prevented reduction of myoplasmic Ca^{2+}. Also, under these conditions ureter muscle generated full sized transient phasic contractions in response to repetitive applications of 20 mmol/l caffeine (Fig. 7*A*). However, readmission of Na_0^+ in spite of the presence of La^{3+} quickly relaxed the tissue and greatly attenuated caffeine contractures (Fig. 7*B*). It was found that cooling significantly slowed the relaxation of the caffeine contractures registered in Na^+, Ca^{2+}-free solution in the presence of La^{3+}. These results

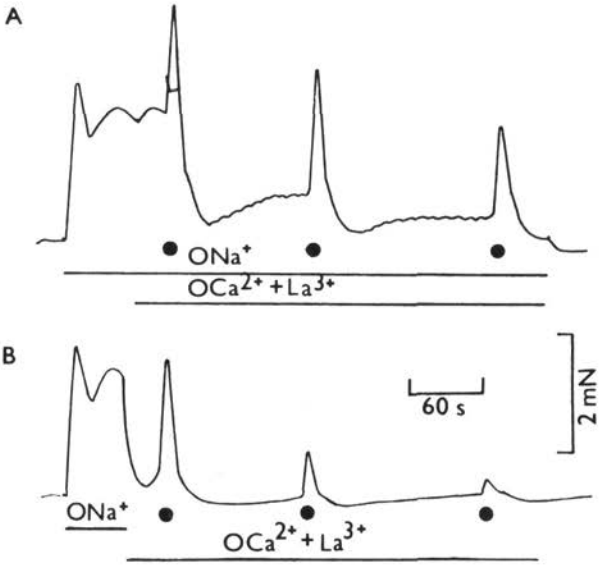

Fig. 7. Tonic tension and phasic contractures induced by caffeine (20 mmol/l) of the Na^+-loaded ureter muscle in Ca^{2+}-free solution with 3 mmol/l-EGTA plus La^{3+} (5 mmol/l) without (*A*) and with (*B*) Na_0^+ readmitted. Note the persistence of tonic tension and caffeine contractures in Ca^{2+}-free La^{3+}-containing solution in the absence of Na_0^+ (*A*) and relaxation of tonic tension and attenuation of caffeine contractures by readmission of Na_0^+ (*B*). The records are taken from two different tissues. Filled circles denote caffeine applications for 20 s.

suggest that when membrane-linked Ca^{2+} extruding systems are inactivated, the contraction and relaxation of the ureter muscle are mediated exclusively by the intracellular Ca^{2+} stores.

Discussion

Assuming that tension development corresponds to an increase in Ca_i^{2+}, the relaxation is likely to be taken as an indicator of the opposite process, i.e. Ca^{2+} removal from the myoplasm. The main conclusion which emerges from the present experiments implies the existence of three major systems which are operating in ureter muscle to regulate cell calcium: SR, Na—Ca exchange and Ca-pump of the cell membrane. The high temperature-dependent system of fast Ca^{2+} uptake which is thought to be directly involved in the relaxation of the phasic contraction is related to SR. The lack of dependence of the rate of relaxation of the phasic contraction on Na_0^+ and Ca_0^{2+} is not consistent with the idea of Na—Ca exchange or Ca^{2+} pump in the cell membrane being rate limiting on the process of the twitch relaxation and therefore the first stage in the removal of Ca_i^{2+}. The time constant of the relaxation of the phasic contraction gives values of 150—250 ms. The time constant of Na—Ca exchange which drives Ca^{2+} in both directions with equal velocity is 10—15 s, i.e. about 100 times higher than the time constant of the twitch relaxation.

Since caffeine which is considered to specifically interfere with Ca^{2+} movement in SR (Weber 1968; Carafoli et al. 1969) produces transient contractures of ureter muscle which has already developed tension induced by either high-K^+ or low-Na^+ solution, it is logical to assume that SR acts as an intracellular Ca^{2+} buffering system in ureter muscle. Accumulation of Ca^{2+} in SR of rabbit portal vein contracted by high-K^+ or noradrenaline was demonstrated by electron probe microanalysis (Bond et al. 1984).

Schwarz (1971) has shown that in the presence of ATP approximately 100 μmol Ca^{2+}/kg of ventricular myocardium can be bound at body temperature within 200 ms (average relaxation time of mammalian ventricles). This means that in fact the rate of Ca^{2+} binding by SR is sufficiently high to completely remove, within one contraction cycle, an amount of Ca^{2+} from the contractile system that is equivalent to that needed for full tension development. Thus, Ca^{2+} binding by SR can be considered to represent the real relaxing mechanism on a short-time scale. Role of SR is accentuated by the ability of ureter muscle to generate full sized repetitive phasic contractures in response to caffeine in Ca^{2+}-free solution when Na—Ca exchange and Ca-pump of the cell membrane are suppressed.

The data obtained suggest that apart from the intracellular Ca^{2+} buffering

system, a system of Na—Ca exchange and Ca-pump of the cell membrane operate in ureter muscle to reduce the myoplasmic Ca^{2+} concentration to such an extent that the intracellular binding sites for Ca^{2+} in SR and myofibrils are kept in a state of rather incomplete saturation. A progressive increase in the baseline tone of the ureter muscle by Na^+-free solution noted earlier (Aickin et al. 1984) and the enhancement of this tone by depolarizing agents which fail to induce any tonic tension in Na^+-containing solution supports the idea of the involvement of Na—Ca exchange in the regulation of cell Ca^{2+}. Dramatic changes in ureter muscle behaviour are caused by elevation of Na_i^+ (Aickin et al. 1984). Elevation of Na_i^+ results in an increase in tonic tension of ureter muscle. Reduction of Na_0^+ under these conditions causes large Ca^{2+} influx resulting in a strong contracture. Our data show that SR only partly opposes this Ca^{2+} influx indicating a limited capacity of this Ca^{2+} buffering system. The Ca^{2+} pump of the plasma membrane is likely to take part in Ca^{2+} extrusion opposing Ca^{2+} influx induced by reversal of the Na—Ca exchanger, since this relaxation is completely blocked by externally applied La^{3+}, which is known to inhibit Ca-pump (Sarkadi et al. 1977). However, La^{3+}-sensitive system of Ca^{2+} extrusion is operating 3—5 times slower than that mediated by Na—Ca exchange which, in fact, proved to be insensitive to La^{3+} as was found in cardiac muscle (Barry and Smith 1982).

References

Aickin C. C., Brading A. F., Burdyga Th. V. (1984): Evidence for sodium-calcium exchange in the guinea-pig ureter. J. Physiol. (London) **347**, 411—430

Barry W. H., Smith T. W. (1982): Mechanisms of transmembrane calcium movement in cultured chick embryo ventricular muscle. J. Physiol. (London) **325**, 243—260

Blaustein M. P., Nelson M. T. (1982): Sodium-calcium exchange: its role in the regulation of cell calcium. In: Membrane Transport of Calcium, (Ed. E. Carafoli) Academic Press, New York, pp. 217—237

Bond M., Shuman H., Somlyo A. P., Somlyo A. V. (1984): Total cytoplasmic calcium in relaxed and maximally contracted rabbit portal vein smooth muscle. J. Physiol. (London) **357**, 185—201

Brading A. F., Burdyga Th. V., Scripnyuk Z. D. (1983): The effects of papaverine on the electrical and mechanical activity of the guinea-pig ureter. J. Physiol. (London) **334**, 79—89

Brading A. F., Sneddon P. (1980): Evidence for multiple sources of Ca for activation of the contractile machinery of the guinea-pig taenia coli on stimulation with carbachol. Brit. J. Pharmacol. **70**, 229—240

Bülbring E., Tomita T. (1969): Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. Proc. Roy. Soc. London Ser. B **172**, 89—102

Carafoli E., Patriarca P., Rossi C. S. (1969): A comparative study of the role of mitochondria and the sarcoplasmic reticulum in the uptake and release of Ca^{++} by the rat diaphragm. J. Cell. Physiol. **74**, 17—29

Casteels R., Raemaekeers L., Droogmans G., Wuytack F. (1985): Na^+—K^+ ATPase, Na—Ca exchange, and excitation-contraction coupling in smooth muscle. J. Cardiovasc. Pharmacol. 7 (Suppl. 3), S103—S110

Goto M., Kimoto Y., Suetsugu Y. (1972): Membrane currents responsible for contraction and relaxation of bullfrog ventricle. Jpn. J. Physiol. **22**, 315—331

Johnishi J., Sunano S. (1978): The role of membrane electrical activities and extracellular calcium in high-K contracture of guinea-pig ureter. Jpn. J. Physiol. **28**, 1—16

Jundt H., Porzig H., Reuter H., Stucki J. W. (1975): The effect of substances releasing intracellular calcium ions on sodium-dependent calcium efflux from guinea-pig auricles. J. Physiol. (London) **246**, 229—253

Kochemasova N. G., Shuba M. F. (1979): Possible ways of extracellular calcium influx necessary for initiation of contraction. In: Physiology and Pharmacology of Smooth Muscle. 2nd International Symposium, Abstracts of Papers, p. 44, Varna

Kuriyama H., Tomita T. (1970): The action potential in the smooth muscle of the guinea-pig taenia coli and ureter studied by the double sucrose-gap method. J. Gen. Physiol. **55**, 147—162

Loutzenhiser R., Leyten P., Saida K., van Breemen C. (1985): Calcium compartments and mobilization during contraction of smooth muscle. In: Calcium and Contractility (Eds. A. K. Grover, E. E. Daniel) The Humana Press, New York, pp. 61—92

Penniston J. T. (1983): Plasma membrane Ca^{2+}-ATPases as active Ca^{2+} pumps. In: Calcium and Cell Function, (Ed. W. Y. Cheung), pp. 99—149, Academic Press Inc., New York

Sarkadi B., Szasz I., Gerloczy A., Gardos G. (1977): Transport parameters and stoichiometry of active calcium ion extrusion in intact human red cells. Biochim. Biophys. Acta **464**, 93—107

Schatzmann H. J. (1982): The plasma membrane calcium pump of erythrocytes and other animal cells. In: Membrane Transport of Calcium. (Ed. E. Carafoli) Academic Press, New York, pp. 41—109

Schwarz A. (1971): Calcium and the sarcoplasmic reticulum. In: Calcium and the Heart. Proceedings of the Meeting of the European Section of the International Study Group for Research in Cardiac Metabolism, London, 1970, (Eds. P. Harris, L. Opie) Academic Press, London—New York, pp. 66—92

Shuba M. F. (1977): The effect of sodium-free and potassium-free solutions, ionic current inhibitors and ouabain on electrophysiological properties of smooth muscle of guinea-pig ureter. J. Physiol. (London) **264**, 837—851

Shuba M. F. (1981): Smooth muscle of the ureter: the nature of excitation and the mechanisms of action of catecholamines and histamine. In: Smooth Muscle. (Eds. E. Bülbring, A. F. Brading, A. W. Jones and T. Tomita), Edward Arnold, London

Somlyo A. P. (1984): Cellular site of calcium regulation. Nature **309**, 516—517

Somlyo A. P., Somlyo A. V., Shuman H. (1979): Electron probe analysis of vascular smooth muscle. J. Cell Biol. **81**, 316—335

Sunano S. (1976): High-potassium induced contracture in guinea-pig ureter. Jpn. J. Physiol. **26**, 717—727

Van Breemen C., Farinas B. R., Casteels R., Gerba P., Wuytack F., Deth R. (1973): Factors controlling cytoplasmic Ca^{2+} concentration. Phil. Trans. R. Soc. London B **265**, 57—71

Washizu G. (1967): Membrane potential and tension in a guinea-pig ureter. J. Pharmacol. Exp. Ther. **158**, 445—450

Weber A. (1968): The mechanism of the action of caffeine on sarcoplasmic reticulum. J. Gen. Physiol. **52**, 760—776

Final version accepted June 19, 1987