Influence of the Sodium Gradient on Contractile Activity in Pregnant Rat Myometrium

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Abstract. The effects of varying the sodium gradient-either by lowering $[Na^+]_o$ or by increasing $[Na^+]_i$ on the electromechanical properties of pregnant rat uterine smooth muscle were studied. In normal tissues, complete removal of external sodium ions (choline, Tris or sucrose as substitutes) induced a strong and maintained contraction which was dependent on the presence of extracellular calcium ions, and was sensitive to Ca^{2+}-antagonist drugs (Nifedipine; D 600, Mn^{2+}). Electrical recordings showed that the membrane was transiently hyperpolarized (-10 ± 2.4 mV, $n = 20$); after 1 minute depolarization accompanied by a spontaneous spike discharge occurred. Partial withdrawal of external sodium ions resulted in following changes in twitch contractions evoked by electrical stimulation: a linear relationship was found between the time constant of twitch relaxation and the external Na-concentration. In Na-rich tissues, where the Na/K pump was blocked, or in the presence of monensin, Na-free solutions (whatever the substitute, even K^+ ions) again triggered strong contractions entirely dependent on external calcium but rather insensitive to Ca-antagonists. The Na-free (K^+) induced contraction was larger than the Na-free (choline or Tris)-induced contraction. It was concluded that the sodium gradient was an important factor for the regulation of contractile activity of uterine smooth muscle. Na-Ca exchange appeared to mediate twitch relaxation in normal tissues and was responsible for Ca-influx in Na-rich tissues.

Key words: Rat myometrium — Na-withdrawal contractions — Twitch relaxation — Na-Ca exchange

Introduction

The contractile activity of mammalian smooth muscles is affected by variations in intra and extracellular sodium concentration (Van Breemen et al. 1979). In

the mouse and rat myometrium, reduction of external sodium between normal and 10% of the physiological concentration increases both amplitude and frequency of spontaneous contractions, while complete removal of sodium ions induces a sustained contraction. These responses seem to be mainly related to calcium influx (Osa 1973; Masahashi and Tomita 1983). Different pathways have been proposed for the entry of calcium ions into smooth muscle cells: i) voltage-sensitive and/or receptor-operated channels (Mironneau 1973; Bolton 1979; Bolton and Kitamura 1983), ii) the reverse mode of Na-Ca exchange mechanism (Reuter et al. 1973), iii) passive influx (membrane leak: Lüllman 1970; Loutzenhiser et al. 1985). Due in part to the difficulties in recording electrical activity in strips (small size of the cells and abundant connective tissue — Gabella 1984), few detailed reports exist on the possible modes of calcium entry in smooth muscles bathed in sodium-deficient solutions. Moreover, a great variety of electromechanical responses are observed in different smooth muscles according to the Na-substitutes, animal species and techniques used (Bülbring and Szurszewski 1974; Kao and McCullough 1975; Takada 1980; Kanda and Kuriyama 1980; Sakamoto and Tomita 1982; Bolton and Kitamura 1983; Aickin et al. 1984). This heterogeneity of smooth muscle activity in solutions with low Na-concentrations may explain the divergence of views on the mechanism of action responsible for these effects, such as Na-Ca exchange. The existence and role of this exchange, especially in vascular tissues, are still a matter of debate (Reuter et al. 1973; Blaustein 1977; Droogmans and Casteels 1979; Aaronson and Van Breemen 1982; Casteels et al. 1985) although this mechanism has long been recognized in some other excitable structures, e.g. squid giant axon (Baker et al. 1969), or cardiac muscle (Reuter and Seitz 1968). In the latter two tissue types the Na-Ca exchange system is voltage-sensitive and electrogenic (Mullins and Brinley 1975; Baker and McNaughton 1976; Horackova and Vassort 1979; Chapman and Tunstall 1980; Coraboeuf et al. 1981). In the myocardium, the participation of Na-Ca exchange in contractile activity has been proved: under physiological conditions it appears to regulate the rate of twitch relaxation (via $[Na^+]_o$ dependent Ca^{2+}-efflux) while at high depolarizations or with a reduced sodium gradient it can mediate influx of calcium ions, which is responsible for the component of tension (Benninger et al. 1976; Roulet et al. 1979; Horackova and Vassort 1979).

The aim of the present paper was to examine and clarify the influence of sodium gradient on calcium movements through the plasma membrane of pregnant rat uterine smooth muscle, paying special attention to possible involvement of the Na-Ca exchange mechanism. We studied the ability of low-Na or high-K solutions to induce contractions, and the effects of the external calcium concentration and of some Ca-antagonists on these responses in normal tissues and in tissues with elevated intracellular Na. The effects of varying the

sodium gradient on the relaxation phase of electrically induced contractions were also investigated.

Materials and Methods

Preparation

Experiments were performed on longitudinal muscle, free of both endometrium and circular muscle, isolated from rat uterus at the end of pregnancy (18—20 days). Strips (60—80 μm in diameter, 3—5 mm in length) and short muscle segments (obtained by crushing the preparation under a grid of fine silver wires) were used for mechanical and electrical recordings respectively. After a stabilizing period (30—60 min) the preparation was used for experimental recordings.

Solutions

Physiological solutions had the following composition: a) reference solution (mmol/l): NaCl 130; KCl 5.6; $CaCl_2$ 2.1; $MgCl_2$ 0.24; glucose 11. The solution was gassed with O_2 and buffered with Tris-HCl (8.3 mmol/l) at pH 7.4. b) in Ca-free solution, $CaCl_2$ was omitted and EGTA added at 5×10^{-4} mol/l. c) low-Na solutions were prepared by substituting equimolar amounts of KCl, Tris-HCl, sucrose or choline (in the presence of atropine 10^{-4} mol/l) for NaCl. d) ouabain (10^{-3} mol/l) and K^+-free solution (KCl omitted) were used to inhibit the Na-K pump activity (Taylor et al. 1970). e) organic compounds (Nifedipine, D 600) or multivalent cations (La^{3+}, Co^{2+}, Mn^{2+}) were used as calcium entry blockers (Mironneau 1973; Fleckenstein 1977). f) in some experiments 5 mmol/l NaN_3 was added in order to inhibit mitochondrial activity.

Mechanical recordings

Isometric contractions were recorded in an experimental chamber which consisted of an open-topped channel, $3 \times 3 \times 20$ mm, connected at one end to a four way-tap opening directly into the channel (Mironneau et al. 1980). The solution entered the channel at a rate of 15 ml min^{-1}. The different solutions were maintained at 35 °C in a thermostated bath. The other end of the channel opened into a drain, so as to avoid perfusion by stagnant solutions. In a distance of about 2 mm from the tap, one end of the strip was fixed to the bottom of the chamber by means of a nylon loop. The other end of the strip was fixed to the lever of a highly sensitive isometric force transducer (Ackers 801, Norway) with a very low drift, good linearity and high sensitivity. The muscle was stimulated either electrically by single electrical pulses (20 ms, 4—5 V) through platinum electrodes located on each side of the chamber, or by perfusion with stimulatory substances (e.g. 10^{-4} mol/l acetylcholine). Control contractions to application of the stimulatory substances were established in reference solution at the beginning of each experiment.

Electrical recordings

Electrical activity was recorded with conventional microelectrodes filled with 3 mol/l KCl (resistance 40—50 MΩ, tip potential less than 10 mV).

Drugs

Chemicals used were: atropine, EGTA, isoprenaline, monensin, ouabain and propanolol (Sigma Chemical Co, St. Louis); phentolamine (Ciba-Geigy, France), Nifedipine (Bayer AG, F.R.G.), D 600 (Knoll F.R.G.).

Expression of the results

The experimental results are expressed as the mean ± S.E. of the mean (S.E.M.) and the number of experiments (n) is given.

Results

I. Effects of sodium withdrawal in control tissues

A. Characteristics of the Na^+-withdrawal contractions

1. Na-free solutions

Switching from the reference solution to a sodium free solution (choline, Tris or sucrose as Na^+ substitutes) induced a large contractile response after a delay of 55 ± 5 s ($n = 30$; Fig 1.*A*). The amplitude of the contraction (1 ± 0.15 mN; $n = 30$) was 3 to 5 times larger than that of the electrically induced twitch and was comparable to that developed in response to a supramaximal dose of

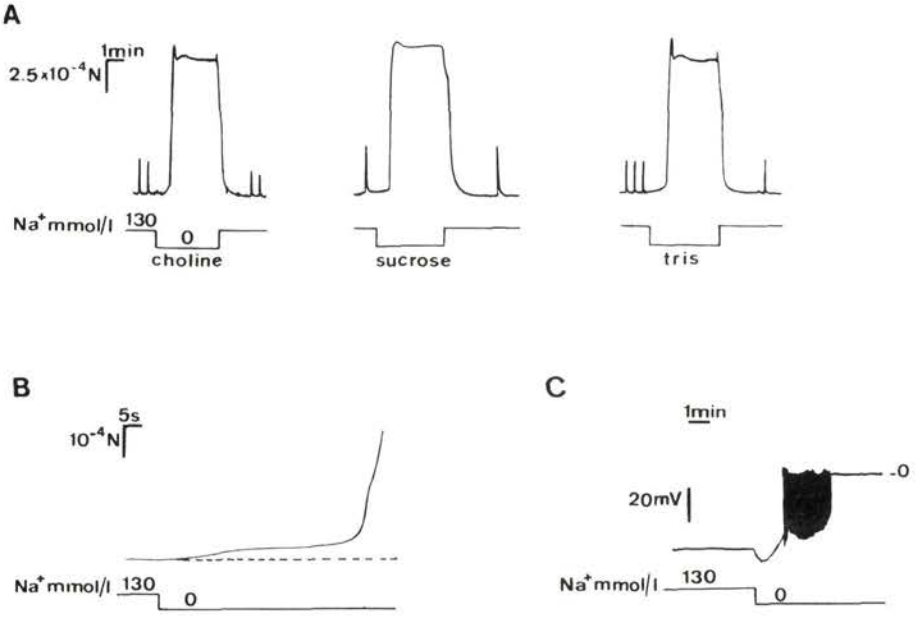

Fig. 1. Effects of Na-free solutions on the electromechanical properties of rat uterine smooth muscle. *A*. Effect of various Na-substitutes on the contractile activity. *B*. Switching to Na-free solution (choline as substitute) induced a biphasic change in the tension. *C*. Electrical activity in the absence of external sodium ions (choline as substitute).

acetycholine (10^{-4} mol/l, not shown). This sodium withdrawal contraction was maintained as long as sodium ions were omitted from the external medium (up to 1 hour). In all cases, on return to the normal sodium concentration 130 mmol/l Na^+), the tissue completely relaxed.

The development of the sodium withdrawal contraction was biphasic. During the delay mentioned previously, a slow increase in resting tension was observed (57 ± 8 μN; $n = 25$ after 50 s) before the appearance of a fast and strong contraction (Fig. 1.*B*). Microelectrode recordings of the electrical activity also showed a biphasic change of the membrane potential in sodium-free solution. The first effect induced by the removal of sodium ions was a transient hyperpolarization of the membrane (10 ± 2.4 mV; $n = 20$) reaching the maximal value in 25 ± 6 s ($n = 20$). After 60 ± 17 s ($n = 20$) the membrane was depolarized and action potentials appeared; the accompanying contraction often displaced the microelectrode (Fig. 1.*C*).

When potassium ions were used as sodium substitute, the contraction (termed potassium contraction) was only 70 ± 4.2 % ($n = 15$) of the sodium-withdrawal contraction (choline or Tris as substitutes and 2.1 mmol/l Ca^{2+} in the external medium) (Figs. 2 and 4), though the membrane was depolarized to -5.2 ± 1.4 mV ($n = 6$). It could be noted that 135 mmol/l K^+-induced contraction did not correspond to the maximal tension obtained when Na^+ ions were progressively replaced by K^+ ions (the maximal K^+ contracture being achieved with 40 mmol/l K^+ — Fig. 2).

2. Partial removal of sodium ions

When choline, Tris or sucrose were the substitute, reduction of $[Na]_o$ to 65 mmol/l Na^+ (50 %) had no effect on the resting tension (Fig. 2). The tension increased with further removal of sodium ions and a close relationship appeared between the tension and the external sodium concentration within 35 to zero mmol/l Na^+. Half maximum contraction was obtained at 13 mmol/l Na^+ (10 % $[Na]_o$). On the other hand, the relationship was observed over a wide range of concentrations when potassium ions were used as Na-substitute (Fig. 2).

3. Effects of $[Ca]_o$ on the Na-withdrawal contractions

The amplitude of the Na-free solution induced contractions was dependent on the external calcium concentration. The Na-withdrawal contraction (choline or

Tris as substitutes) appeared more sensitive to extracellular calcium ions than the potassium contraction. The half maximum responses were achieved at 0.28 and 1.2 mmol/l Ca^{2+} respectively (Fig. 3). Whatever the Na^+ substitute, no contractile response could be evoked when the muscle strip was bathed in a calcium-free EGTA (0.5 mmol/l)-containing solution.

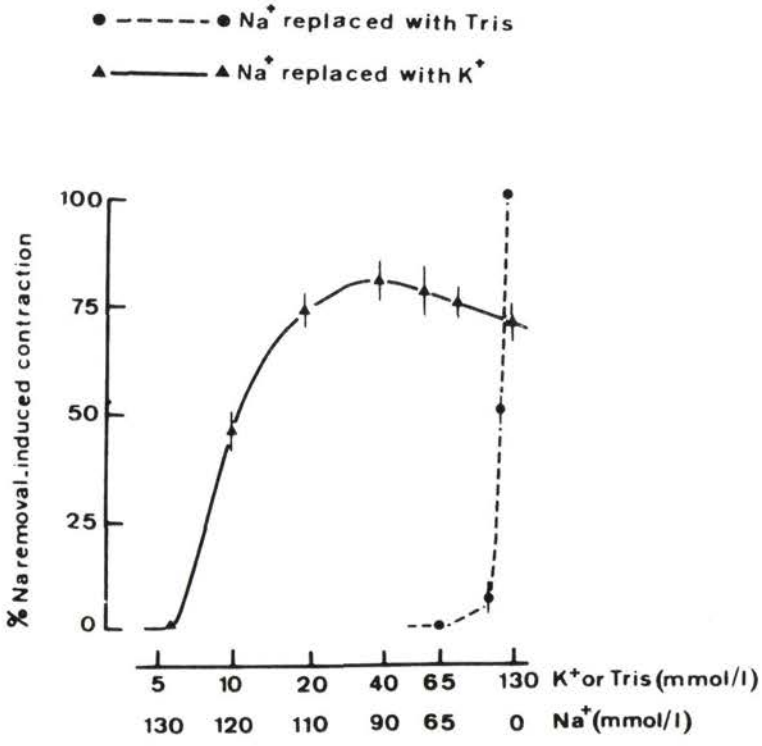

Fig. 2. Effects of progressive replacement of sodium ions by K^+ (▲—▲) or Tris (or choline ●—●) in the presence of 2.1 mmol/l Ca^{2+}. Points are means ± S.E.M. of seven experiments. For the curve ●—●, each point is the mean of four experiments with Tris and three experiments with choline as Na-substitutes.

4. Effects of Ca-antagonists

The above results suggested that influx of calcium ions was the main factor responsible for the development of contractions in sodium deficient solutions.

Table 1. Concentrations of Ca-antagonist drugs which produce 50% reduction of the Na-free solution-induced contraction.

Na-substitute	Drug (mol/l)		
	Nifedipine	D 600	Mn^{2+}
Choline or Tris	5×10^{-9}	3×10^{-7}	1.1×10^{-3}
K^+	6×10^{-10}	1.4×10^{-7}	4×10^{-4}

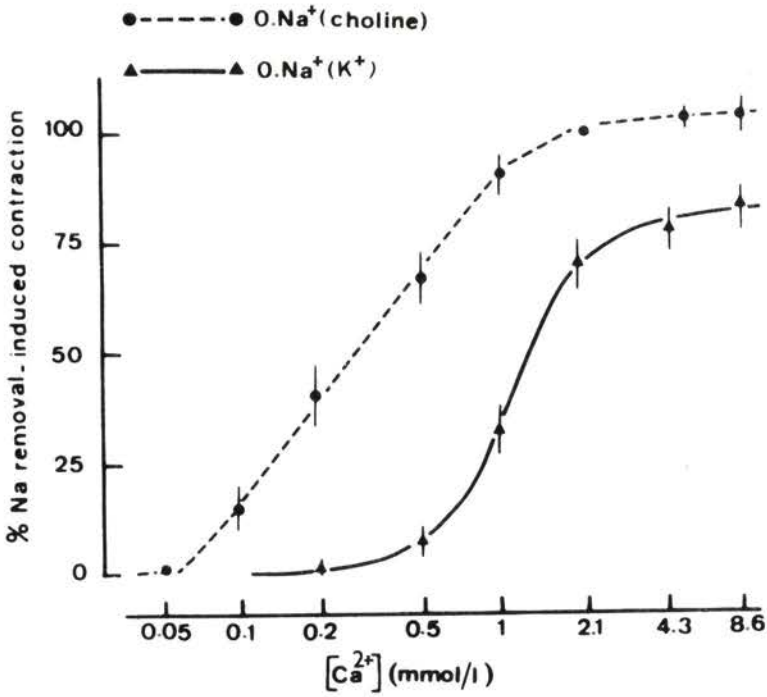

Fig. 3. Dose-response curves for the effect of external calcium concentration on the amplitude of the Na-free solution-induced contracture. ●---● choline as substitute, ▲—▲ K^+ as substitute. Points are means ± S.E.M. of seven experiments. The value of the Na-free (choline)-induced contracture in the presence of 2.1 mmol/l Ca^{2+} is taken as 100%

Therefore, the effects of some Ca-antagonistic drugs were tested on these contractile responses. Nifedipine, D 600 and Mn^{2+} ions inhibited the Na-withdrawal contraction in a dose-dependent manner (Fig. 4 and Table 1). It could be noticed that high doses of organic calcium antagonists did not completely

suppress the sustained contraction. The remaining component of the maximal Na-withdrawal contraction, insensitive to nifedipine and D 600 was $15 \pm 4\%$ and $18 \pm 5\%$ ($n = 5$) respectively (Fig. 4.*C*). When K^+ was the Na-substitute a complete inhibition was obtained at 2.5×10^{-9} mol/l Nifedipine and 10^{-6} mol/l D 600.

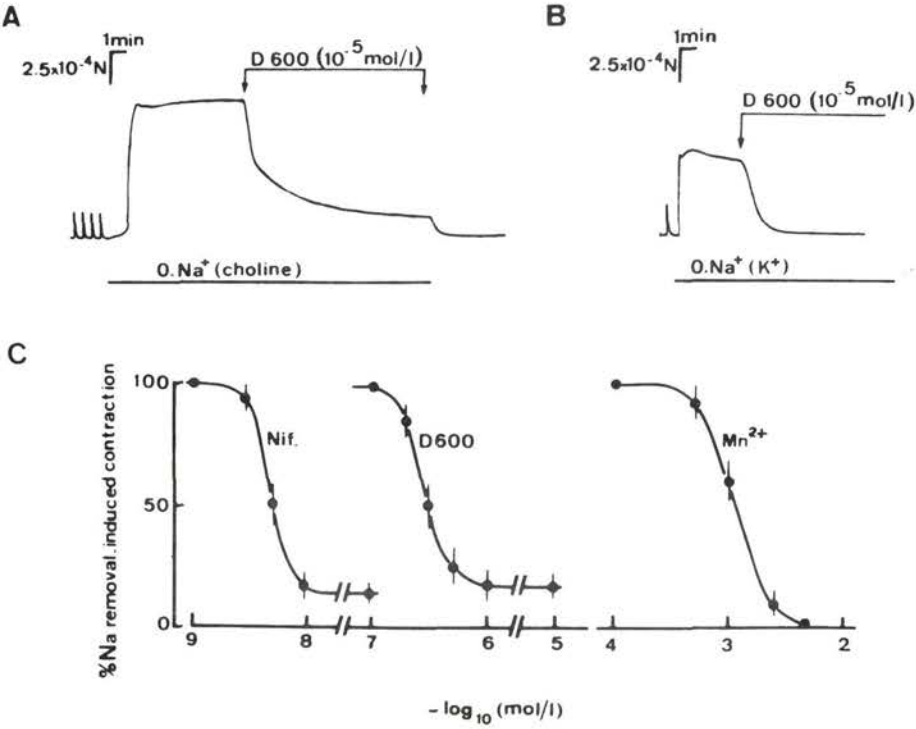

Fig. 4. Effects of Ca-antagonist drugs on the Na-free solutions-induced contractions. *A*. Effect of D 600 (10^{-5} mol/l) on the Na-free(choline)contraction. *B*. Effect of D 600 (10^{-5} mol/l) on the Na-free(K^+) contraction. *D*. Dose-response curves for the effect of nifedipine (Nif.), D 600 and manganese ions (Mn^{2+}) on the amplitude of the Na-free(choline) contraction. Points are means $\pm$ S.E.M. of five experiments. The value of the Na-free(choline)-induced contraction in the presence of 2.1 mmol/l Ca^{2+} is taken as 100%.

B. Effect of sodium withdrawal on twitch relaxation

1. Twitch characteristics

In reference solution (130 mmol/l Na^+) a single electrical pulse (20 ms, 4—5 V) elicited a transient contractile response (twitch): its amplitude, time to peak and

duration (measured as the time interval between the activation and the relaxation phase when the tension declined to 10% of its maximal amplitude) were $2.7 \times 10^{-4} \pm 0.6 \times 10^{-4}$ N; 1.1 ± 0.3 s; 10.2 ± 2.7 s ($n = 30$) respectively. The semi-logarithmic plot of the decaying phase showed that the relaxation was well fitted by a single exponential function with a time constant of 2.8 ± 0.6 s, $n = 30$ (Fig. 5.*A*).

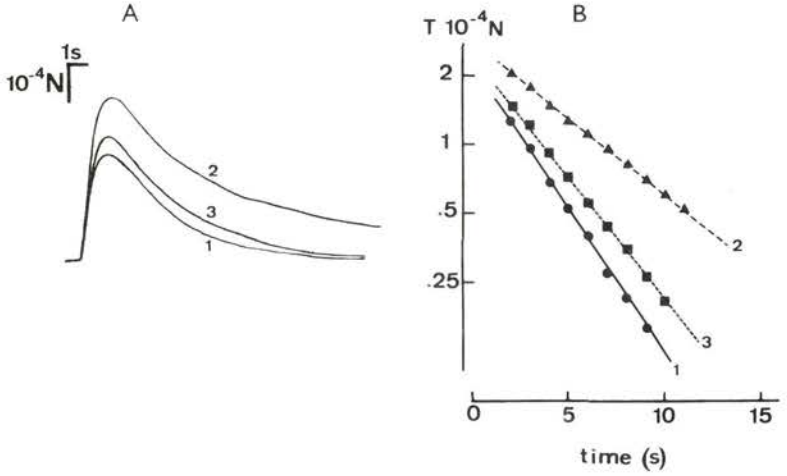

Fig. 5. Effect of a 50% reduction (65 mmol/l Na^+) of the external sodium concentration on the twich relaxation. *A*) Superimposed traces of twitches recorded in reference solution (1), after 8 min. in the Na-deficient solution (2) and 5 min. after return to normal Na-containing solution (3). *B*) Semi-logarithmic plots of the relaxation phase of twitches: 1. reference solution, $\tau = 3$ s; 2. 65 mmol/l Na^+, $\tau = 5.4$ s; 3. recovery, $\tau = 3.2$ s.

2. Effects of lowering $[Na]_o$

When $[Na]_o$ was reduced to 65 mmol/l (a 50% reduction, choline as substitute) no modification of the resting tension was observed (Fig. 2), but the amplitude of the twitch was generally increased and the relaxation was slowed down. After 8 min in Na-deficient solution, the time constant (τ) increased to 5 ± 0.7 s, $n = 6$ (Fig. 5). On readmission of the reference solution, the initial parameters of the twitch were recovered in a few minutes. As previously shown, further reduction of $[Na]_o$ was characterized by an increase in the resting tension and the development of a sustained contraction causing the twitches to disappear. In order to prevent these responses, the temperature of the perfusing solutions was lowered to below 30 °C (28 ± 0.5 °C). In these experimental conditions, low Na^+-containing solutions induced only a moderate contraction upon which twitches could be triggered. Fig. 6 shows a typical record in Na-free

solution: after a transient contraction, twitches were elicited and their duration progressively increased because the relaxation dramatically slowed down. In this experiment τ increased from 2.6 s in reference solution to 9 s after 15 minutes in Na-free solution.

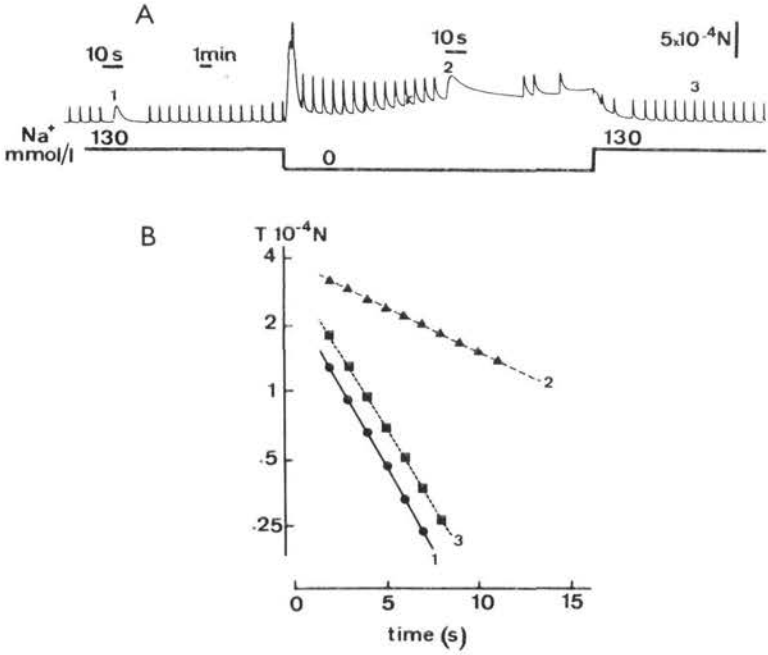

Fig. 6. Effects of complete removal of Na^+ ions on the twitch. Experiment was performed at 28 °C. A) Recording of the mechanical activity of uterine smooth muscle strip. Each twitch was elicited by a single electrical pulse (4 V, 20 ms). In the absence of external Na^+ ions, the relaxation phase was very slowed (compare 1 and 2). B) Semi-logarithmic plots of the relaxation phase of twitches: 1. reference solution, $\tau = 2.6$ s; 2. Na-free solution, $\tau = 9$ s; 3. recovery (8 min.), $\tau = 2.7$ s.

A linear relationship between the reduction in $[Na]_o$ and the time constant of twitch relaxation was observed (Fig. 7).

II. Effects of increasing $[Na]_i$

The intracellular sodium content of smooth muscle cells can be raised in various ways, e.g. i) by addition of monensin, a cation selective ionophore which transports Na^+ at a faster rate than K^+ (Pressman and Fahim 1982; Ozaki et al. 1984), ii) by blockade of the Na-K pump by K-free solutions and/or addition of ouabain (Taylor et al. 1970; Ozaki et al. 1978; Aickin et al. 1984). In rat

tissues, high doses of ouabain are required to fully inhibit the Na/K pump which has a low sensitivity to the glycoside (Beaugé and Ortiz 1970). In smooth muscle cells of rat resistance vessels, application of 10^{-3} mol/l ouabain for 30 minutes increases $[Na]_i$ 3 times (Aalkjaer and Mulvany 1983; Mulvany et al. 1984).

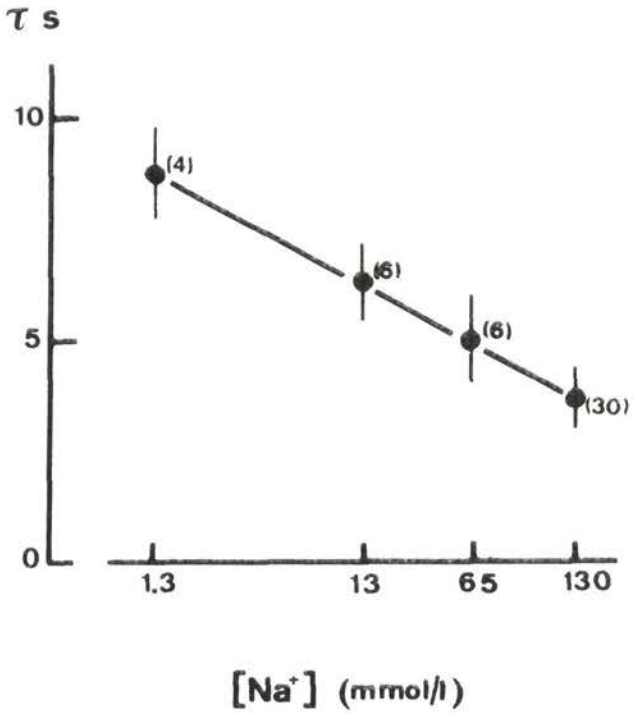

Fig. 7. Relationship between the external sodium concentration ($[Na]_o$) and the time constant (τ) of the twitch relaxation. The points are means ± S.E.M. with the number of experiments indicated in parentheses. (Abscissa: logarithmic scale).

A. Contractile responses

When one of these procedures was applied to uterine smooth muscle, typical changes in the mechanical activity were observed. Generally, spontaneous tetanus was evoked during the first minutes (Fig. 8.*A*; K^+ free-solution) then rhythmic contractions together with an increase in tension appeared. After 15—20 minutes rhythmic contractions fused in a sustained contraction which was maintained as long as K^+ ions were omitted. On return to the reference solution, the muscle progressively relaxed (Fig. 8.*A*). Similar effects were obser-

ved with monensin (10^{-4} mol/l) and ouabain (10^{-3} mol/l); the former acted more rapidly and the latter more slowly, the maximum amplitude of the sustained contracture being achieved in 12.3 ± 2.2 min with monensin; in 30.6 ± 5.5 min with K^+-free solution and in 43.5 ± 4.2 min with ouabain ($n = 5$).

The conctractile responses were not modified either by the presence of adrenoreceptors-blocking agents (phentolamine 3×10^{-6} mol/l, propranolol 3×10^{-6} mol/l) or by the presence of a muscarinic receptor antagonist (atropine 10^{-4} mol/l). The inhibitor of mitochondrial functions NaN_3 (5×10^{-3} mol/l) had no effect either.

B. Effects of Ca-antagonists and of external Ca concentration

Blockage of the electrogenic sodium pump or rapid inflow of sodium ions induced by monensin may depolarize uterine smooth muscle cells (Mironneau et al. 1982) and consequently activate calcium voltage-sensitive channels which could be responsible for the observed sustained contraction. This hypothesis

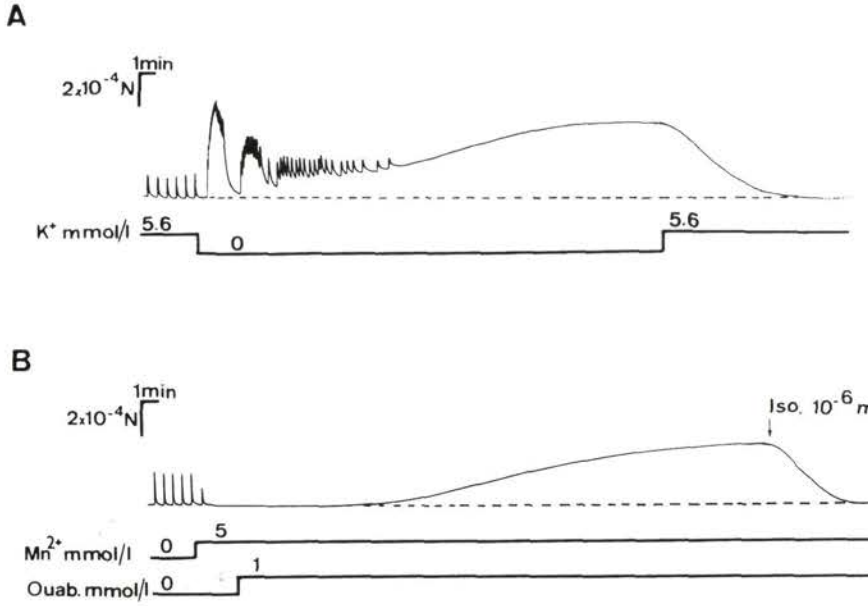

Fig. 8. Effects of increasing $[Na]_i$ on the contractile activity of the uterine smooth muscle. *A*. Effect of a K^+-free solution. *B*. Ouabain (1 mmol/l) induced a contraction even in the presence of 5 mmol/l Mn^{2+} ions (a concentration which fully abolished twitches and tetanus). Isoprenaline (ISO — 10^{-6} mol/l) antagonized the ouabain-induced contraction. Dotted lines indicate the value of the resting tension.

was tested by the addition of some calcium antagonists. High concentrations of nifedipine (10^{-8} mol/l), D 600 (10^{-6}—10^{-5} mol/l) or Mn^{2+} ions (5 mmol/l) actually suppressed tetanus and rhythmic contractions but exhibited only moderate inhibitory effect on the sustained contractions (25.4 ± 4.2%; 19 ± 3.7% and 22.4 ± 4.3%, $n = 5$ respectively) (Fig. 8.*B*).

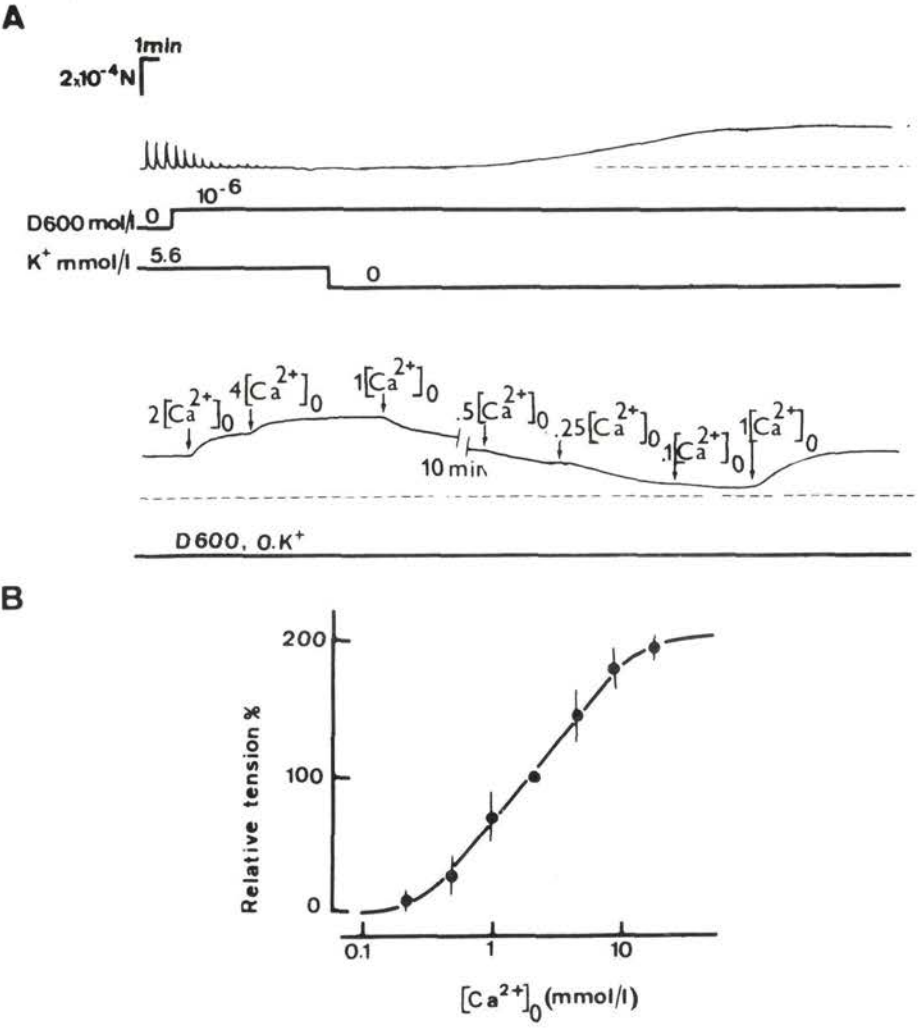

Fig. 9. Effects of external calcium concentration ($[Ca^{2+}]_o$) on the Ca-antagonists-resistant contraction in high-Na tissues. *A*. In the presence of D 600 (10^{-6} mol/l), a K^+-free solution induced a slow contraction the amplitude of which was dependent of the external calcium concentration. *B*. Dose-response curve for the effect of external Ca-concentration on the amplitude of the K^+-free solution-induced contraction. Points are means ± S.E.M. of five experiments. The response obtained for 2.1 mmol/l Ca^{2+} is taken as 100%.

Nevertheless, the tension remaining insensitive to calcium antagonists was totally abolished by isoprenaline (10^{-6} mol/l) and appeared sensitive to variations in the external calcium concentration (Fig. 9.*A*). The amplitude of this component of tension appeared as a sigmoid function of $[Ca]_o$ (Fig. 9.*B*). In calcium-free solution (containing 0.5 mmol/l EGTA), blockade of the Na-K pump or addition of monensin produced no contractile response in pregnant rat uterine smooth muscle.

C. Effects of increasing $[Na]_i$ on the twitch relaxation

During the first 5—10 minutes of K-free/ouabain or monensin treatment, it was possible to induce twitches. Fig. 10 shows an example of a twitch 4 min after the addition of 10^{-4} mol/l monensin. Both resting tension and amplitude increased while the rate of relaxation decreased. In this example τ increased from 3 to 5.5 s.

Identical effects were observed with ouabain (10^{-3} mol/l) or K^+-free solution.

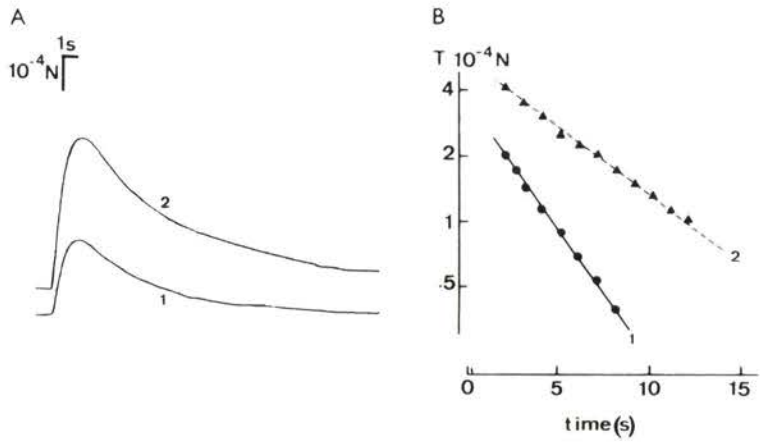

Fig. 10. Effect of increasing $[Na]_i$ on twitch relaxation. *A*) Superimposed traces of twitches recorded in reference solution (1), and 4 min. after the addition of 10^{-4} mol/l monensin (2). *B*) Semi-logarithmic plots of the relaxation phase. 1: reference solution, $\tau = 3$ s; 2: with monensin, $\tau = 5.5$ s.

III. Effect of sodium removal in high-Na-tissues

A. Electromechanical responses

In a subsequent series of experiments, tissues were incubated in ouabain (10^{-3} mol/l) for 1 hour, then sodium was removed from solutions. In order to

limit the participation of the contraction described above, calcium ions were omitted during the ouabain treatment and reintroduced just before the withdrawal of external sodium ions. The possible participation of voltage-sensitive calcium channels was ruled out by the presence of 10^{-6} mol/l D 600. As previously shown (Fig. 4) the Na-withdrawal contraction (choline or Tris as substitutes) was reduced to 18% of the maximum response by the organic Ca-antagonist while after ouabain treatment it reached $75 \pm 7\%$ ($n = 10$) of its maximal response (Fig. 11.*A*).

In the same experimental conditions, microelectrode recordings showed that the Na-withdrawal induced hyperpolarization was not modified by D 600. It was again transient, with the same peak amplitude (10.5 ± 1.6 mV, $n = 10$) as observed earlier and was followed by a small membrane depolarization of 4.2 ± 0.8 mV ($n = 10$). Action potentials were abolished (compare Figs. 11.*B* and 1.*C*). Sodium readmission induced an additional depolarization of 8.5 ± 1.5 mV ($n = 10$) which slowly declined. After ouabain treatment, the

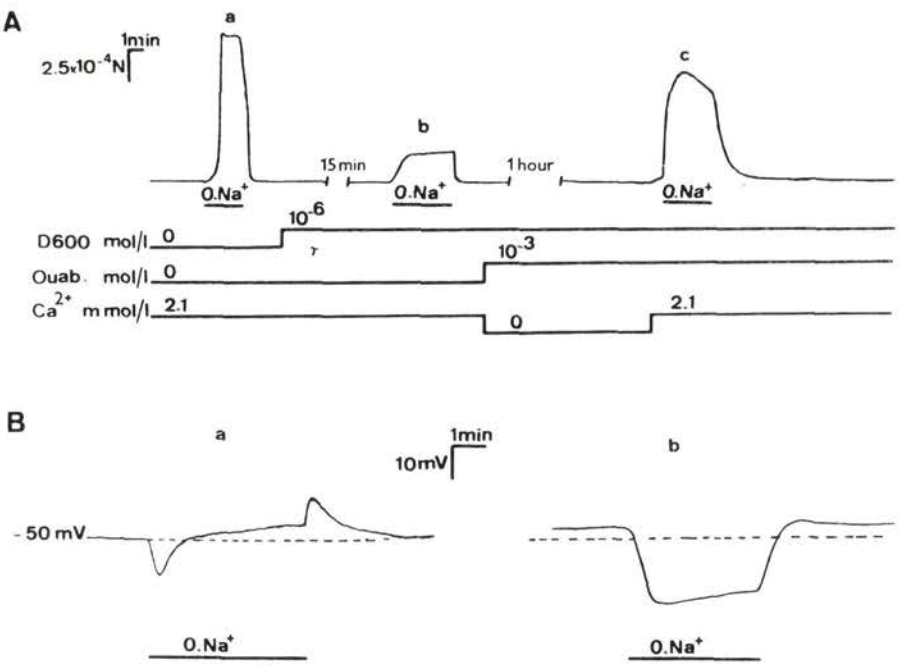

Fig. 11. Electromechanical responses induced by sodium removal in high-Na tissues. (Preparations were pretreated with ouabain 10^{-3} mol/l for 1 hour). *A*. Na-removal-induced contraction in reference solution (a), in the presence of D 600 alone (b), and ouabain (c). *B*. Membrane potential changes induced by Na-removal in the presence of D 600 (10^{-6} mol/l) before (a) and after (b) ouabain-treatment.

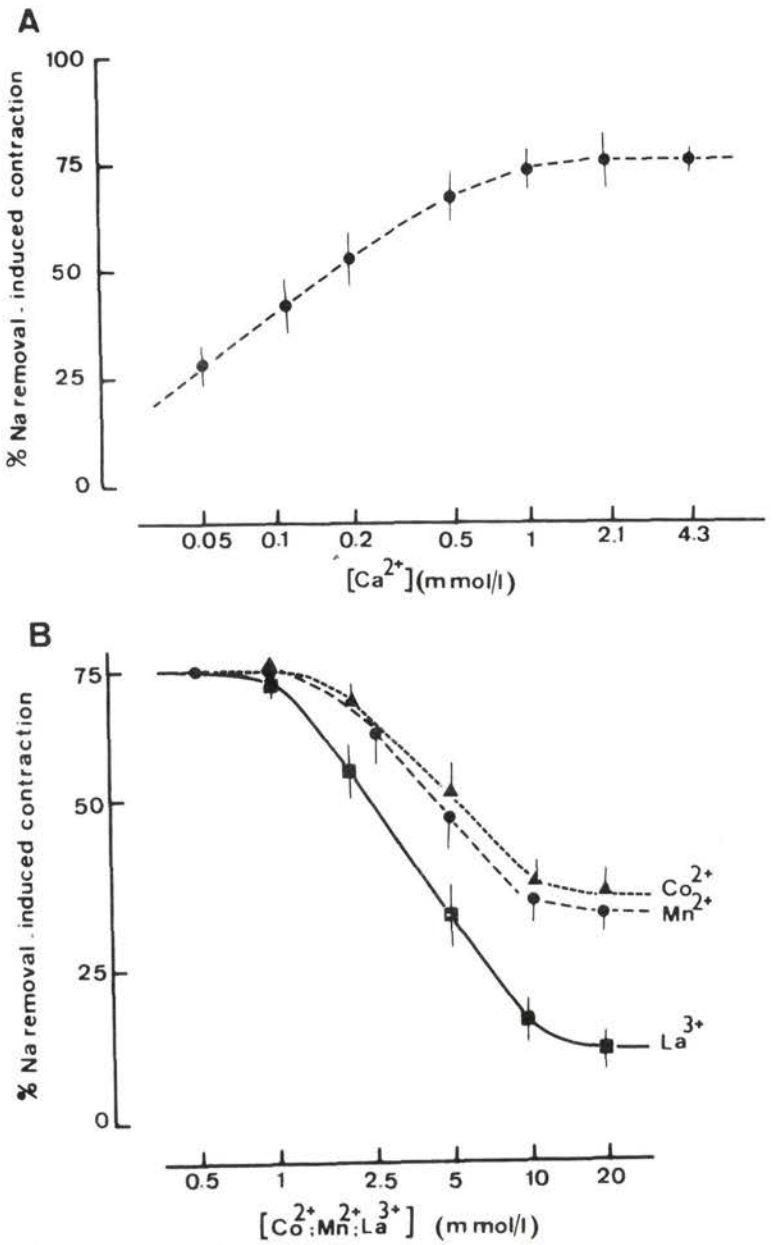

Fig. 12. Sensitivity to external calcium concentration and to multivalent cations of the Na-removal induced contraction in high Na-tissues. (Preparations were pretreated with ouabain 10^{-3} mol/l for 1 hour). *A*. Dose-respone curve for the effect of $[Ca^{2+}]_o$ on the Na-free (choline) contraction. *B*. Dose-response curve for the effect of cobalt (Co^{2+}), manganese (Mn^{2+}) and lanthanum (La^{3+}) ions on the Na-free (choline) contraction, in the presence of 2.1 mmol/l Ca^{2+}. Points are means $\pm$ S.E.M. of five experiments. Control response obtained in normal tissues with 2.1 mmol/l Ca^{2+} is taken as 100%.

membrane was generally slighty depolarized by 3.8 ± 1.4 mV ($n = 5$) in the presence of 2.1 mmol/l Ca^{2+}. At this time, Na-withdrawal produced a larger and sustained hyperpolarization reaching a peak amplitude of 19.5 ± 2.3 mV ($n = 5$), (Fig. 11.*B*).

B. Effects of both external Ca concentration and multivalent cations

After ouabain treatment, the sodium withdrawal contraction was more sensitive to low concentrations of extracellular calcium (cf. Figs. 12.*A* and 3). The concentration required to produce half maximum response was 0.08 mmol/l Ca^{2+}, and the response appeared to be saturated at 1 mmol/l Ca^{2+}. The Na withdrawal contraction in Na-rich tissues became very resistant to multivalent cations known as Ca^{2+}-antagonists. High concentrations (10—20 mmol/l of Co^{2+}, Mn^{2+} and La^{3+}) strongly but not completely inhibited this contraction (Fig. 12.*B*). Similar phenomena were observed when K^+ ions were the Na-substitute though the depolarization was not modified by ouabain treatment.

More rapid and more spectacular events appeared when ouabain and monensin were added together in the external medium. Fig. 13. shows that after

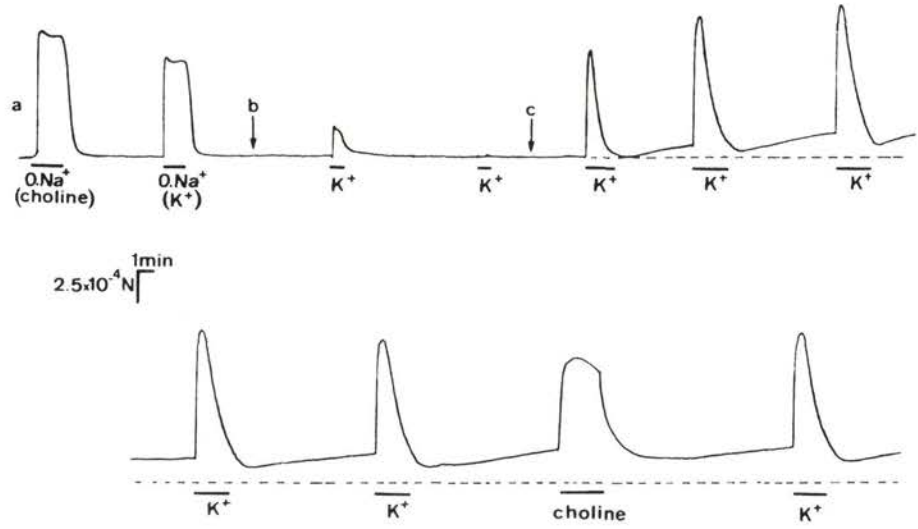

Fig. 13. Repeated contractile responses induced by sodium removal in high-Na tissues. *a*) Control responses to application of a Na-free solution (choline or K^+ as substitutes) were established in the reference solution. *b*) Addition of 10^{-7} mol/l nifedipine abolished the K^+-induced contraction. *c*) In the presence of nifedipine, ouabain (10^{-3} mol/l) and monensin (10^{-4} mol/l) were simultaneously added to the bathing solution. After 3 min of this treatment K^+-contraction reappeared and could be evoked repeatedly for 1 hour.

total inhibition of K^+-contraction by a supramaximal dose of nifedipine (10^{-7} mol/l), the perfusion of the muscle strip with 10^{-3} mol/l ouabain and 10^{-4} mol/l monensin caused reappearance of the contraction within 3 min. Then, repeated application of the 135 mmol/l K^+ solution evoked repeated transient contractions the amplitude of which increased and became higher than that of normal tissue ($126 \pm 8\%$ $n = 15$ of the maximal response in normal tissue). Interestingly, it could be noted that the response was smaller ($104 \pm 5\%$ $n = 8$) but more sustained when choline was the Na-substitute.

All these responses were unaffected by 5 mmol/l NaN_3, and vanished in Ca-free EGTA (0.5 mmol/l) containing solutions.

Discussion

The present investigation shows that the contractile activity of the pregnant rat myometrium becomes altered in several ways when the transmembrane sodium gradient is modified. On reducing this gradient, either by lowering $[Na]_o$ or by increasing $[Na]_i$, the twitch relaxation is slowed down and generally the resting tension increases. In Na-free solutions, the longitudinal uterine muscle develops strong contractions which are always entirely dependent on extracellular calcium but exhibit different properties according to the cellular sodium content. These results suggest that several mechanisms of calcium transport with variable efficiency are involved in these responses.

In normal tissues with low $[Na]_i$, about 15—20 mmol/l (Hamon et al. 1976; Kishimoto and Urakawa 1982), complete sodium removal (choline, Tris or sucrose as substitutes) triggers a sustained contraction which appears mainly correlated with a continuous discharge of spikes (Fig. 1). In smooth muscles, sodium and calcium ions are known to compete for anionic sites on the outer membrane surface and thus to control the membrane potential (Brading et al. 1969; Tomita and Watanabe 1973). Membrane depolarization and action potentials in Na-deficient solutions have also been observed in mice myometrium (Osa 1973) guinea pig stomach (Ohba et al. 1977; Sakamoto and Tomita 1982), and rat portal vein (Yamamoto and Hotta 1986).

In rat myometrium, the electrical activity has been shown to be of the calcium type in both, muscle strips (Mironneau 1974; Vassort 1975; Jmari et al. 1986) and isolated cells (Mollard et al. 1986). Moreover, a close relationship between the amplitude of the calcium inward current and that of one component of tension has been established in the uterus (Mironneau 1973). For these reasons, it is obvious that the sodium-withdrawal contraction in normal tissues is mainly due to calcium influx through voltage-sensitive channels. Nevertheless, some other mechanisms may be activated by the removal of sodium ions (e.g membrane leak or reversal in the Na-Ca exchange mechanism). As shown in

Fig. 1.*B.C*, a slow increase in the resting tension occurs during the first minute after switching to Na-free solution whereas the membrane is transiently hyperpolarized at the same time. Different factors may be involved in this hyperpolarization: i) reduction of inward Na- background current, ii) the reverse mode of electrogenic Na-Ca exchange, iii) secondary stimulation of some Ca^{2+}-dependent potassium channels (Mironneau and Savineau 1980; Savineau et al. 1984). In the presence of D 600, the hyperpolarization is still induced and followed by a small depolarization (Fig. 11.*B*). This D 600-resistant depolarization which normally brings the membrane potential to the spike threshold may be due to a simple diffusion of calcium ions. Such a direct pathway insensitive to organic Ca-antagonist drugs has been described in other smooth muscle preparations (Casteels and Droogmans 1981; Suzuki et al. 1982; Lalanne et al. 1984; Loutzenhiser et al. 1985).

When K^+ ions are used as the Na-substitute, the contraction is obviously triggered by influx of calcium ions through voltage-sensitive channels. However, in agreement with Gabella's data (1978), the amplitude of the 135 mmol/l K^+-induced contraction is not the maximum tension evoked by K ions and it is only 70% of the maximum response obtained with other substitutes (Fig. 2 and 4). Such a discrepancy may result from a change in Ca-channel properties in high K^+-medium, as shown in giant axon (Strickholm 1981), and/or from the activation of different populations of calcium channels as shown in the development of K^+-contractions in ileum (Hurwitz et al. 1980) and cerebral arteries (Högestätt and Anderson 1984).

After ouabain treatment (10^{-3} mol/l for 1 hour) the sodium withdrawal contracture (choline, Tris or sucrose as substitutes) becomes more resistant to calcium antagonist drugs and is related to a larger and more sustained hyperpolarization of the membrane (Fig. 11.*B*). The fact that the contraction is totally dependent on external Ca-concentration and increases as a function of the external Ca suggests that calcium is still entering from the outside but through another way than by voltage-sensitive channels. Thus, some Na-Ca countertransport mechanism appears to be a putative system to account for these phenomena. From the values reported in other studies (Kishimoto and Urakawa 1982; Petersen and Mulvany 1984; Mulvany et al. 1984) and the effect observed on twitch relaxation in this work (Fig. 10., and compare with the effect of 50% reduction of $[Na]_o$) it can be assumed that $[Na]_i$ increases at least threefold in these tissues. The removal of extracellular sodium ions may produce a large outward sodium gradient that in turn may trigger influx of calcium ions ($[Na]_i$-dependent Ca-influx). Similarly, sodium free solutions also induce simultaneous hyperpolarizations and contractions poorly affected by calcium entry blockers (D 600, Mn^{2+}) in Na-rich cells of dog Purkinje fibers (Coraboeuf et al. 1981) and guinea pig ureter (Aickin et al. 1984).

When ouabain and monensin are simultaneously added to the perfusing solution, these effects are more rapidly generat 1 (Fig. 13). Interestingly, in these experimental conditions the potassium-induced contraction is larger and more transient than the choline or Tris-induced contraction. Since Na-substitution by potassium is accompanied by a depolarization, an enhanced Ca entry would be expected if Na-Ca exchange were sensitive to voltage. A possible additional Ca entry through potential-sensitive Ca channels is ruled out by the presence of Ca-antagonists (e.g 10^{-7} mol/l nifedipine) which totally abolish the K-contractions in normal tissues (Table 1). As the membrane is depolarized in Na-free (K^+) solution and hyperpolarized in Na-free (choline) solution, the difference in size of the contractions could be explained by the possibility that the Na-Ca exchange is electrogenic. It is known that depolarization enhances and hyperpolarization diminishes the $[Na]_i$-dependent Ca influx in the giant axon (Baker and McNoughton 1976; Mullins and Requena 1981; Di Polo et al. 1982) and in cardiac muscle (Horackova and Vassort 1979; Allen et al. 1983).

In some tissues such as nerve terminals of the rabbit pulmonary artery (Török et al. 1984) variations of $[Na]_i$ produce release of calcium ions from internal Ca-stores. In the mammalian heart, the participation of mitochondria in the low Na^+ induced contractile responses has been questioned (Chapman et al. 1983; Chapman 1986) and related to the presence of Na-Ca exchange located on the external mitochondrial membrane (Crompton et al. 1976; see for review Carafoli 1985). No similar study has been carried out on smooth muscles and no significant modifications in the uterine responses have been observed in the presence of 5 mmol.l^{-1} NaN_3.

The existence of an electrogenic Na-Ca exchange mechanism in the plasma membrane of rat myometrium is also suggested by the following observations: i) in normal tissues and in the presence of D 600, readmission of sodium ions to Na-free solution induces a fast relaxation of the D 600-resistant contraction and an additional and transient depolarization of the membrane (Fig. 11). ii) blockade of the Na/K pump or addition of monensin produce a slow increase in the resting tension. This moderate contraction is rather insensitive to Ca antagonists, though absent in Ca-free solution (Fig. 9). Increasing $[Na]_i$ might lead to a reduction of sodium gradient-dependent Ca efflux and/or the activation of $[Na]_i$-dependent Ca-influx. A clear relationship between the increase in $[Na]_i$ (after blockade of the Na-K pump) and the amplitude of tension has been shown in guinea-pig and rat aorta (Ozaki and Urakawa 1981). iii) the clear effect of the sodium gradient on the relaxation phase of the twitch. A close relationship appears between the value and the direction of the sodium gradient and the time constant of twitch relaxation (Fig. 7). When the Na gradient is reversed in normal tissues, τ is amplified by a factor of 3—4. As previously mentioned, twitch of rat uterine smooth muscle is triggered by a calcium action potential

(Mironneau 1973; Amédée et al. 1986) which produces an increase in the cytoplasmic calcium concentration. Obviously, the relaxation follows the recovery of initial $[Ca]_i$ (to about 10^{-7} mol/l; Endo et al. 1977). Whatever the internal processes of sequestration, an ultimate extrusion of calcium ions should occur as a consequence of the Ca influx during action potential. Two mechanisms are generally proposed for the sarcolemmal transport of calcium in smooth muscle cells: an ATP-dependent Ca-pump (Casteels and Van Bremen 1975; Wuytack et al. 1985) and Na-Ca exchange. The observation that with a 50% reduction of $[Na]_o$ (65 mmol/l) the resting tension is not modified whereas the twitch relaxation is already slowed down (Fig. 5) leads us to assume that in normal tissues, the Na gradient is not the primary mechanism for regulating $[Ca]_i$ at rest but it is essential when $[Ca]_i$ has been previously increased. The lack of difference in the time constant of twitch relaxation between 35 °C and 28 °C also favours the predominance of Na-Ca exchange in the relaxation process.

A similar dependence of twitch relaxation on the sodium gradient occurs in amphibian cardiac muscle (Roulet et al. 1979) where, as in smooth muscles, the cells have a high surface/volume ratio and a poorly developed sarcoplasmic reticulum.

In conclusion, our study shows that the sodium gradient is an important factor in the regulation of the contractile activity of rat uterine smooth muscle, especially when the cellular sodium content is high. Most of the effects observed in Na-rich tissues can be explained by the operation of an electrogenic sarcolemmal Na-Ca exchange. In normal tissues (with a low $[Na]_i$) this exchanger is required for the twitch relaxation to occur, but other mechanisms are involved in the regulation of $[Ca]_i$ at rest and in the development of the Na-free sustained contractions.

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