

## Possible Role of Na<sup>+</sup> Ions in Intracellular Ca<sup>2+</sup> Release in Frog Auricular Trabeculae\*

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**Abstract.** Membrane potential-current and mechanical tension of frog atrial muscle were studied in a Ca and Mg-free solution containing 1 mmol/l EGTA (Ca-free solution).

1. Exposure to Ca-free solution resulted in a shortening of action potential duration within 1.5 min and a subsequent lengthening which were paralleled by changes in magnitude and duration of the contraction.

2. Similarly, the slow inward current quickly disappeared and progressively reappeared with a quite slower inactivation time-course. Its reversal potential varied with [Na]<sub>o</sub> as for a pure Na current.

3. By 12 min in Ca-free solution, the tension-voltage relation could be interpreted as the sum of two components correlated with the slow inward current and the membrane potential respectively.

4. Contractures in response to sustained large depolarizations had similar time courses in Ca-free solution and Ringer's containing Na-Ca exchange blockers (Mn<sup>2+</sup> 15 mmol/l or La<sup>3+</sup> 3 mmol/l).

5. Intracellular Na loading by voltage-clamp depolarizations (40 mV from the resting potential for 100 ms, at 0.2 Hz) in the presence of Veratrine (7.5 × 10<sup>-6</sup> g/ml) caused a large progressive increase in tonic tension. An intracellular Ca<sup>2+</sup> release is invoked, partly related to Na<sup>+</sup> entry and partly to membrane potential changes. The potential dependent part could be influenced by intracellular Na<sup>+</sup>.

**Key words:** Sodium ions — Intracellular calcium release — Excitation-contraction coupling — Frog myocardium

### Introduction

It is generally believed that the frog heart mechanical activity elicited by depolarization consists of two components which mainly depend on extracellular calcium: i)

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phasic tension related to the calcium inward current (Goto et al. 1971; Vassort and Rougier 1972; Léoty and Raymond 1972; Einwächter et al. 1972), and ii) tonic tension (Vassort 1973; Benninger et al. 1976) which seems to depend mainly on a voltage dependent sodium-calcium exchange mechanism (Horačkova and Vassort 1979a).

However, in a recent work, Horačkova and Vassort (1979b) have suggested that the phasic tension is regulated by two mechanisms: the slow inward current and release of  $\text{Ca}^{2+}$  from intracellular stores. The latter would be a Ca-induced Ca release phenomenon.

We observed that frog atrial preparations equilibrated with a Ca-free solution respond to a large depolarization by generating tension transients as large as and of longer duration than those in control medium containing 2.5 mmol/l  $\text{CaCl}_2$ .

In Ca-free solution, the calcium inward current (Rougier et al. 1969) and the Na-Ca exchange mechanism (Horačkova and Vassort 1979a) are strongly depressed and an entry of  $\text{Ca}^{2+}$  ions on depolarization is unlikely to occur. Thus, the tension transients may be attributed to a release of  $\text{Ca}^{2+}$  ions from intracellular stores, triggered by a mechanism other than Ca-induced Ca release.

The present study intended to provide some insight into the coupling mechanism(s) between depolarization and this intracellular Ca release.

A preliminary report has already been published (Bonvallet et al. 1980).

## Materials and Methods

### a) Preparations and apparatus:

The experiments were carried out at room temperature (20 to 24 °C) on trabeculae (50–100  $\mu\text{m}$  in diameter, 3–4 mm in length) isolated from the atrium of the frog *Rana esculenta*. The sucrose gap layout and the electronics used for measurements under current clamp and voltage clamp conditions were the same as described earlier by Rougier et al. (1968) and more recently by Garnier et al. (1978).

### b) Solutions:

The control solution (Ringer's) had the following composition (in mmol/l): NaCl 110; KCl 2.5;  $\text{CaCl}_2$  1.8;  $\text{MgCl}_2$  2; Tris-HCl buffer 10. Chemicals used to prepare solutions were of high grade purity (MERCK) chosen for their low divalent cationic contaminants. All solutions were prepared extemporaneously with twice deionized water and, once all components had been dissolved, pH was adjusted to 7.4 by adding appropriate amounts of HCl, NaOH or KOH accordingly. The calcium-free solution was obtained by omitting calcium and magnesium salts and by adding a chelating agent: EGTA (ethyleneglycol-bis(-amino-ethylether)-N, N, N', N'-tetraacetic acid), at a concentration of 1 mmol/l. This lowered  $[\text{Ca}^{2+}]_o$  below the resting intracellular level (Linden and Brooker 1982) without making the cell membrane excessively leaky (Miller and Mörchen 1978). Solutions with modified  $[\text{Na}^+]_o$  were obtained by isoosmotic substitution of sucrose for NaCl. These Na-depleted solutions have the advantage of reducing the phenomena of accumulation-depletion of  $\text{K}^+$  ions within the clefts (Ojeda et al. 1982). Thus, the leakage current is more stable since its time dependence due to  $[\text{K}^+]_o$  variations is greatly reduced. However, they have the disadvantage of increasing the resistance of the extracellular fluid, thereby somewhat reducing the accuracy of voltage control, which is, nevertheless, still satisfactory during the flow of the slow inward current.

In few experiments (see the text), TTX ( $10^{-6}$  mol/l) or Veratrine ( $7.5 \times 10^{-6}$  g/ml) were used. Isotonic mannitol (240 mmol/l) was used as the insulating solution, its conductivity was raised to  $1 \mu\text{S/cm}$  by adding  $\text{CaCl}_2$  to prevent cell decoupling (Kléber 1973).

*c) Voltage-clamp measurements:*

Both at the beginning and end of each experiment, the resting potential of the preparation was estimated under current clamp conditions as the change in potential recorded when changing the  $\text{K}^+$  concentration of the solution flowing in the test compartment from 2.5 to 125 mmol/l (Ojeda and Rougier 1974). When the value was less than 70–80 mV, or when it dropped by more than 10 % along the experiment, results were not considered.

Holding potential (HP) is the potential at which the membrane of the fibre was maintained.  $\text{HP} = 0$  mV corresponds to the resting potential (estimated to be  $-80$  mV in Ringer solution), the latter being the potential at which no net current flows (zero current potential).  $V$  (mV) is the variation of membrane potential from HP and is noted positive for depolarizations and negative for hyperpolarizations. Currents are noted positive when outward, negative when inward.

In the presence of TTX ( $10^{-6}$  mol/l), in order to block completely the fast inward sodium current (Rougier et al. 1969; Connor et al. 1975), the slow inward current was measured as the difference between the downward peak value and the value of the current at the end of a pulse of 100 to 200 ms in duration. For potentials around +40 mV, the slow inward current may be slightly underestimated because it is not completely inactivated at the end of the pulse. The delayed outward current should not greatly influence this measurement since it is only weakly activated for pulses up to 200 ms in duration (Ojeda and Rougier 1974).

The time interval between successive voltage pulses or action potentials was at least 4 s.

*d) Contraction recording:*

Contraction of portion of the preparation in the test gap was recorded with an electromechanical transducer (RCA 5734: Vassort and Rougier 1972; or Endeveco 8101: Chapman 1970). Its response is linear between 0 and 200 mg.

Each result reported on was obtained in 3 to 5 separate preparations and the recordings shown here were representative of the whole series. No precise numerical values are to be retained since this study did not aim to be more than qualitative.

## Results

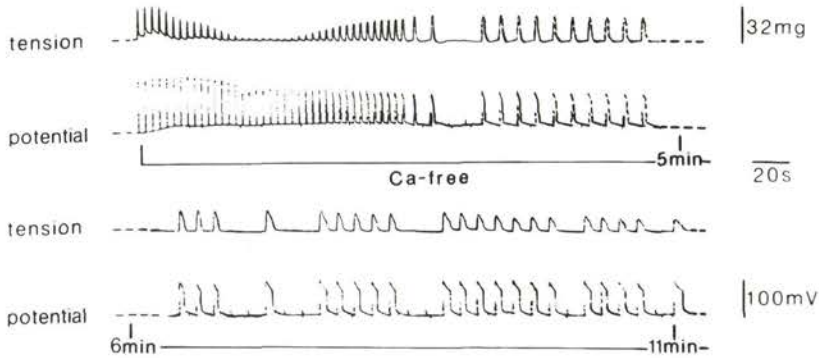
### *A — Effects of Ca-free solution on action potential and contraction*

The preparation was stimulated at 0.25 Hz in control solution and we checked that the action potential and contraction records were stable in shape for at least 5 min (not shown) before changing to Ca-free solution.

The effects of Ca-free solution (e.g. Fig. 1) on the electrical activity may be summarized as follows:

— The resting potential diminished by about 25 mV from its initial value within about half a minute and remained at this level throughout the exposure (e.g. 11 min in Fig. 1);

— the amplitude of the overshoot of the action potential decreased by about 30 mV within the first minute and remained at this level throughout the experiment;



**Fig. 1.** Effects of Ca-free solution on action potential and contraction. Notice that, even after 11 min, an action potential may elicit a contraction. Because of the large increase in action potential duration, the rate of stimulation was decreased from 0.25 Hz to 0.1 Hz after 2 min 30 s.

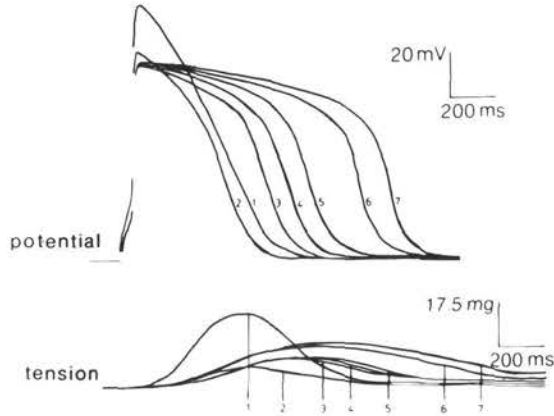
— the action potential duration increased significantly beyond 1 to 2 min in this medium.

These electrical modifications were accompanied by mechanical changes. Switching to Ca-free solution almost immediately caused a decrease in contraction amplitude. After about 1 min, the amplitude was reduced to one tenth of its control value, but after 1 to 2 min it started to increase progressively. Altogether, action potential and contraction durations were increasing almost in parallel. After 2 to 3 min, the amplitude of the contraction reached that in control and remained at this level for about 2 min before slowly decreasing again. After about 10 min, contraction amplitude was reduced by about 50%. Throughout the experiment, the diastolic tension was not significantly modified.

The changes in action potential and contraction during the first minutes of exposure to Ca-free solution are illustrated in Fig. 2: passing from control solution (trace 1) to modified solution, caused first a 10% decrease in the duration of the action potential (measured at 90% repolarization) and a 20 mV decrease in the amplitude of its overshoot (trace 2). This initial transient effect was followed by a progressive increase in action potential duration (traces 3–7) which was approximately doubled within 3 min.

During the first phase (about 30 s), contraction amplitude decreased by about 75% and its time to peak remained almost unchanged. During the second phase, the amplitude increased to about half the control amplitude and the time to peak rose up to twice the control one in about 4 min (traces 2–7).

The increase in duration of the contraction closely followed the increase in action potential duration.



**Fig. 2.** Biphasic evolution in the action potential and contraction during the first minutes in  $\text{Ca}$ -free solution. 1: control solution; 2 to 7: 30 s; 1 min 30 s; 2 min; 2 min 30 s; 3 min; and 3 min 45 s in  $\text{Ca}$ -free solution, respectively. For clarity, resting potentials levels were artificially superimposed. Interval between two successive action potentials was 5 s.

The results concerning the action potential are quite comparable to those of Garnier et al. (1969). However, the results concerning contraction differ from those of Miller and Mörchen (1978) who observed that tension could no longer be detected soon after the change of solutions.

Even if the inotropic effect may, at first sight, seem directly related to changes in the action potential, voltage clamp experiments were performed:

- to try to explain the modifications of the action potential;
- to examine the link(s) between action potential and tension development.

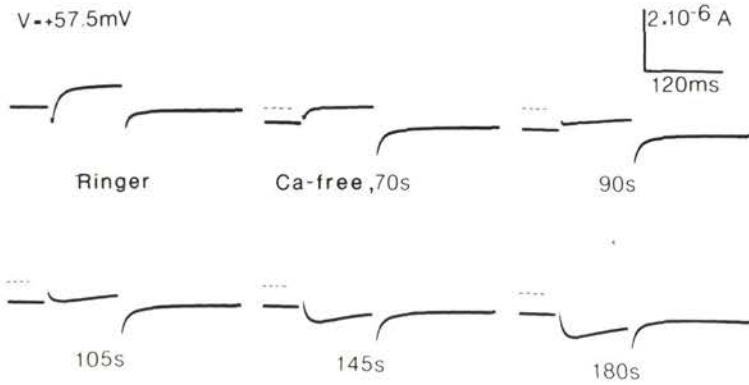
#### *B — The slow inward current in $\text{Ca}$ -free medium: its relation to mechanical events.*

##### *a) Modifications of the slow inward current during the first minutes of exposure:*

Figure 3 shows recordings of the slow inward current, in the presence of TTX ( $10^{-6}$  mol/l); the preparation was depolarized by 57.5 mV for 115 ms in normal Ringer's, and after different times in  $\text{Ca}$ -free solution. This amplitude of depolarization was chosen because it used to activate a nearly maximal slow inward current in control medium.

In normal Ringer's, this current is carried both by calcium and sodium ions (Rougier et al; 1969). The time constant of its inactivation was about 30 ms for  $V$  near 60 mV.

As soon as calcium and magnesium ions had been removed from the superfusing solution, the holding current was shifted in the inward direction (dashed line on  $\text{Ca}$ -free solution recordings corresponds to the holding current level in control solution); it reached a steady level after 1 to 2 min and remained at



**Fig. 3.** Biphasic evolution in the slow inward current for a 57.5 mV depolarization for 115 ms, during the first minutes in Ca-free solution. The dashed line, on Ca-free recordings, corresponds to the resting background level in control solution. TTX  $10^{-6}$  mol/l was present throughout.

this level throughout the exposure. The slow inward current decreased, and after 1.5 to 2 min, it was almost suppressed. Then, after about 2 min, an inward current reappeared; its amplitude increased with time and its inactivation seemed much slower than it was the case in control. For the short duration of the pulse used, the current was not completely inactivated at the end of the pulse. For pulses longer than 250 ms (not shown), the current reached a steady level at the end of the pulse; its inactivation was 3–4 times slower than in normal Ringer.

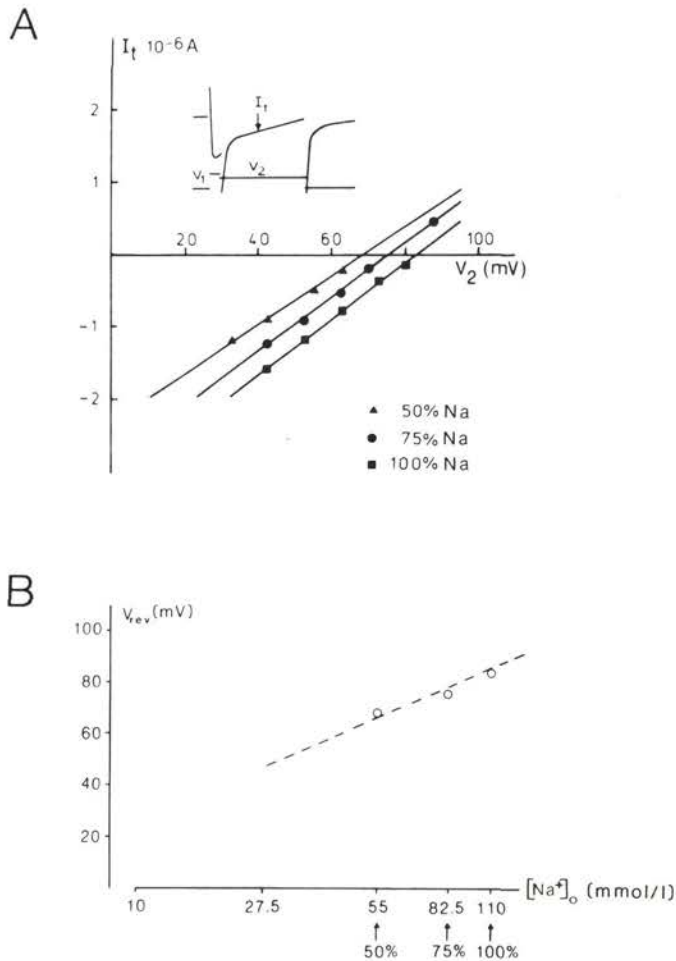
#### b) Ionic nature of the slow inward current in Ca-free solution:

It is generally admitted that the slow inward current in Ca-free solution is a pure sodium current which is not inhibited by TTX (Rougier et al. 1969; Garnier et al. 1969).

To confirm this result in our conditions, we determined the apparent reversal potential of the slow inward current for three external sodium concentrations (in mmol/l: 110, i.e. 100 % Na; 82.5, i.e. 75 % Na; 55, i.e. 50 % Na) using the double pulse method (Reuter and Scholz 1977). TTX ( $10^{-6}$  mol/l) was added to block the fast initial sodium current. Each curve was established after 5 min in the test solution.

Figure 4A shows the instantaneous current-voltage relationships obtained by double pulse experiments. The apparent reversal potential was shifted by  $-8$  and  $-15$  mV when  $[Na^+]_o$  was lowered to respectively 75 and 50 % of its control value.

In Fig. 4B, the apparent reversal potentials, as determined in A, were plotted versus the logarithm of the external sodium concentration. These experimental points are almost located on a straight line with a slope of 58 mV per 10-fold variation in  $[Na^+]_o$ , as expected from the Nernst equation at 22 °C. This seems to

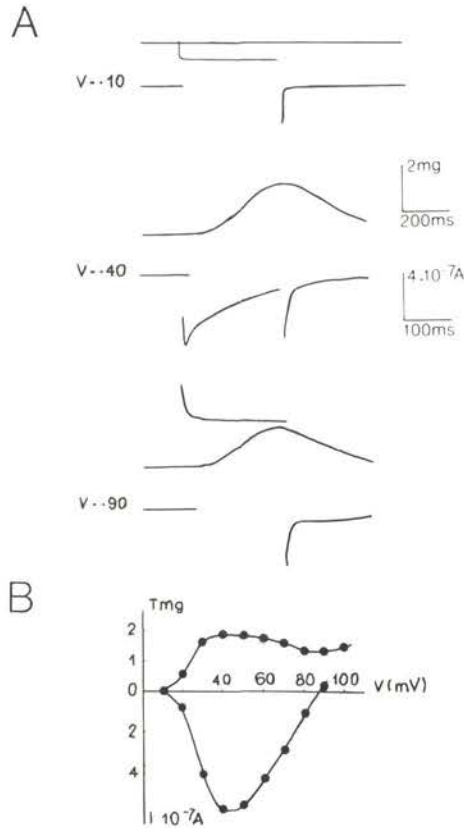


**Fig. 4.** A: determination of the apparent reversal potential of the slow inward current in Ca-free solution for three external sodium concentrations, using the double pulse method (see inset).  $V_1$  is a 40 mV depolarizing pulse of 26 ms duration; the tail currents corresponding to the different values of  $V_2$  (depolarizing test pulse of 210 ms in duration) were analyzed in a semi-logarithmic plot versus time: their initial amplitude ( $I_{t=0}$ ) thus determined was plotted versus amplitude of  $V_2$ . B: apparent reversal potential values determined in A, plotted versus the logarithm of the external sodium concentration. Note that the experimental values are almost located along a straight line with a slope of 58 mV per decade.

confirm that the slow inward current in Ca-free solution behaves like a pure sodium current.

c) Relation between slow inward sodium current and mechanical activity:

Figure 5A shows recordings of the contraction and current elicited by three

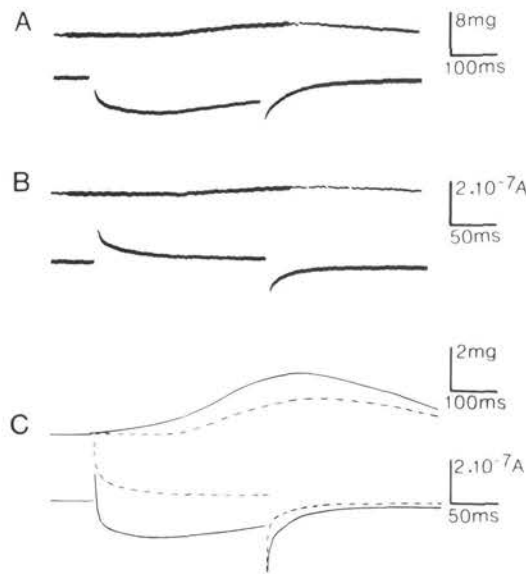


**Fig. 5.** Relation between slow inward sodium current and mechanical activity. **A:** slow inward sodium current and contraction obtained for three depolarizations of 210 ms in duration after 12 min in a 75 % Na (sucrose substitute) Ca-free solution containing  $10^{-6}$  mol/l TTX. **B:** slow inward sodium current-voltage and tension-voltage relations obtained on the same preparation as in **A**. The current was measured as the difference between the downward peak value and the value of the current at end of pulse.

depolarizations of 210 ms duration after 12 min in 75 % Na, Ca-free solution containing TTX ( $10^{-6}$  mol/l). All the results are summarized in the tension-voltage and current-voltage curves (Fig. 5B). The thresholds for tension and current development were similar; both relationships showed maximal values for a depolarizing step of about 40 mV. Then, both slow inward current and tension decreased for larger depolarizations. At 90 mV depolarization, the slow inward current was zero and the tension showed a relative minimum. For larger depolarizations, the curve relating tension to voltage raised again.

We also tested the influence of  $Mn^{2+}$  ions (3 mmol/l) upon the electrical and



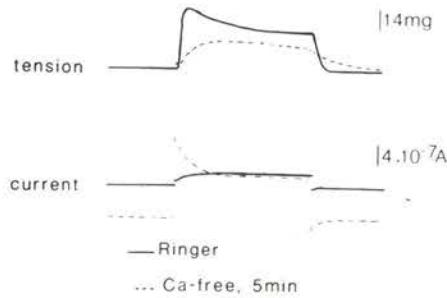


**Fig. 6.** Influence of  $\text{Mn}^{2+}$  ions (3 mmol/l) on the electrical and mechanical responses induced by a 57.5 mV, 190 ms depolarizing step in a 75 % Na (sucrose substitute) Ca-free solution containing  $10^{-6}$  mol/l TTX. A: control solution; B: 55 s after addition of  $\text{Mn}^{2+}$  ions; C: superimposition of records A (solid line) and B (dashed line). For clarity, the contraction records were amplified.

mechanical responses induced by a depolarizing step about 60 mV in amplitude and 200 ms in duration.

The recording in Fig. 6A was obtained after 11 min 30 s in the same medium as before. 55 s after the addition of  $\text{Mn}^{2+}$  ions (Fig. 6B), the slow inward sodium current was suppressed. Nevertheless, a contraction could still be recorded but its amplitude decreased by 40 % as compared to that in control solution.

Thus, the mechanical response in Ca-free solution seems, at least partly, to be related to the slow inward sodium current and partly to the polarization of the membrane. Nevertheless, at this stage, the action of some external  $\text{Ca}^{2+}$  ions cannot be ruled out. If the diffusion of EGTA into intercellular clefts were incomplete, some calcium ions might remain and enter the cell through the Na—Ca exchange mechanism. This might explain the increase in tension obtained with depolarizing steps above the reversal potential for  $I_{\text{si}}$  (Fig. 5) and the remaining tension recorded in the presence of 3 mmol/l  $\text{Mn}^{2+}$  (Fig. 6B). In order to test this possibility, we performed contracture experiments in a calcium containing medium in the presence of Na—Ca exchange blockers:  $\text{Mn}^{2+}$  15 mmol/l (Chapman and Ellis 1977; Ellis 1977; Horačkova and Vassort 1979a; Coraboeuf et al. 1981) or



**Fig. 7.** Effects of Ca-free solution on contracture induced by a command pulse of 100 mV in amplitude and 12 s in duration. For further explanations, see text. Note that contracture was recorded after 5 min in Ca-free solution.

$\text{La}^{3+}$  3 mmol/l (Horačkova and Vassort 1979a) and in a Ca-free solution without blocker.

#### C — Contracture experiments:

##### a) Effects of Ca-free solution on contracture induced by sustained depolarization:

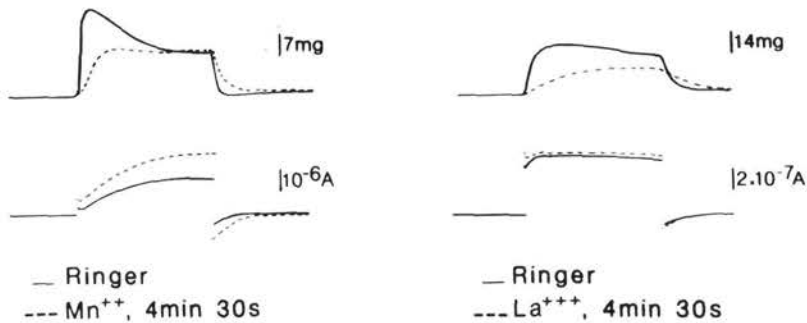
From  $\text{HP} = 0$  mV, a test depolarization of 100 mV was applied for 12 s (Fig. 7).

In normal Ringer (solid lines), as the depolarization was established, a large contracture (upper traces) was elicited, rapidly reaching maximum and then slowly decreasing to a steady level. After the end of the pulse, the tension rapidly returned to the previous resting level. During the depolarization, an outward current (lower traces) developed up to a steady value.

After about 5 min in Ca-free solution (dashed lines), a similar depolarization induced a very different contracture. It slowly reached a maximum and then remained at this maximum throughout the depolarization, after the end of which it slowly returned to the resting level. As compared to the control one, the current recorded in this medium showed a large inward shift in holding current and, in response to the depolarization, a large initial surge of outward current appeared which decreased to a steady value similar to that in control.

##### b) Effects of $\text{Mn}^{2+}$ and $\text{La}^{3+}$ ions on contracture induced by sustained depolarizations in normal Ringer:

The recordings of Fig. 8 show the effects of  $\text{Mn}^{2+}$  ions (15 mmol/l) (left recordings, dashed line) and of  $\text{La}^{3+}$  ions (3 mmol/l) (right recordings, dashed line) 4 min 30 s after their addition to normal Ringer. Tension (upper traces) and current (lower traces) were recorded in response to depolarizations of 100 mV in



**Fig. 8.** Effects of  $\text{Mn}^{2+}$  and  $\text{La}^{3+}$  ions on contracture induced by a command pulse of 100 mV in amplitude and 12 s in duration in calcium containing medium. For further explanations, see text. Note that, even in the presence of Na—Ca exchange blockers, a contracture could be elicited.

amplitude and 12 s in duration from  $\text{HP} = 0$  mV. These results were obtained in two different preparations.

In each case, a similar depolarization induced a contracture which was quite different from the control one (full line). These modified contractures, as in Ca-free solution, slowly reached a maximum and remained at this level throughout the depolarization, at the end of which they relaxed more slowly than in normal Ringer.

The outward current was increased twofold in the presence of  $\text{Mn}^{2+}$ , but only slightly in the presence of  $\text{La}^{3+}$ .

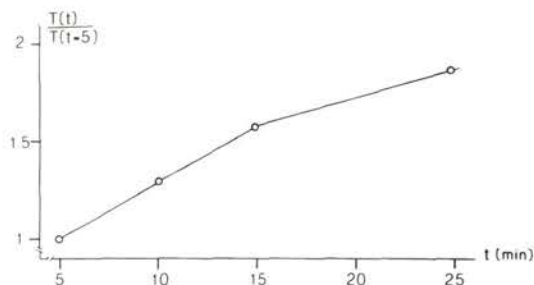
After 5 min washout in normal Ringer following either  $\text{Mn}^{2+}$  or  $\text{La}^{3+}$  exposure, the contracture recovered a fast onset, its initial peak was partly restored and its steady level returned to the control one (not shown). The current trace returned to control shape.

These contracture experiments suggest that the tonic component recorded in Ca-free solution might be due to a release of  $\text{Ca}^{2+}$  ions from some internal store(s), probably induced by membrane potential changes.

In order to test whether this "voltage-dependent tonic component" could be influenced by intracellular  $\text{Na}^+$  concentration, we performed experiments in the presence of an agent, Veratrine, known to increase intracellular sodium concentration (Horačkova and Vassort 1974).

### c) Effects of increasing the intracellular sodium concentration on tonic tension in Ca-free solution:

The preparations were stimulated by depolarizing clamp steps of 40 mV in amplitude and 100 ms in duration at 0.2 Hz in Ca-free solution, containing Veratrine (7.5 g/ml). This compound prevents cardiac fast Na channels from inactivating (Horačkova and Vassort 1974) and thus large amounts of  $\text{Na}^+$  will



**Fig. 9.** Evolution with time of the mechanical activity recorded in Ca-free solution containing Veratrine ( $7.5 \times 10^{-6}$  g/ml). For further explanations, see text.

enter the cells on depolarization, in addition to  $\text{Na}^+$  entering through the slow inward channel. The Na—K pump will fail to draw it out (Horačkova and Vassort 1974). At different times during the loading procedure, a test depolarization of 100 mV in amplitude and 12 s in duration was applied.

The results are shown in Fig. 9. The  $T(t)/T(t=5)$  ratio (ordinate) represents the amplitude of the mechanical activity elicited by the test depolarization after  $t$  min in Ca-free solution + Veratrine, divided by the amplitude of the mechanical activity in response to an identical depolarization after a 5 min exposure. The amplitude was measured at the end of the test depolarization. The increase in amplitude of the contraction was regular throughout the 25 min exposure.

## Discussion

When a frog atrial trabecula was superfused with a Ca-free solution, its electrical activity underwent several changes: the resting potential and the amplitude of the overshoot of the action potential decreased (Fig. 1). The action potential duration first decreased and then progressively increased (Fig. 2). These results are quite comparable to those previously described on the same preparation (Rougier et al. 1969; Garnier et al. 1969) and on frog ventricle (Miller and Mörchen 1978; Linden and Brooker 1982).

By contrast to the results that Miller and Mörchen (1978) obtained on frog ventricle superfused with a OCa, Omg, EGTA (0.2 mmol/l) solution, our results show that mechanical activity can be obtained even after 11 min (Fig. 1) in a OCa, Omg solution containing 5 times more EGTA than it was the case in experiments of the above authors.

Linden and Brooker (1982) calculated that their calcium-free solution,

containing 1 mmol/l EGTA, would have a free  $\text{Ca}^{2+}$  concentration of  $1.7 \times 10^{-9}$  mol/l. In our Ca-free solution, the pH-buffer was not the same as used by the above authors. However, Miller and Mörchen (1978) measured a free  $\text{Ca}^{2+}$  concentration of  $2.2 \times 10^{-8}$  mol/l in a solution having a salt composition very similar to that of ours, including pH-buffer and containing only 0.2 mmol/l EGTA. Thus it is reasonable to apply the result of Linden and Brooker's calculation to our solution.

After 11 min in Ca-free solution, the extracellular spaces of our small preparations, even if EGTA did not easily diffuse into them, will have been depleted of their  $\text{Ca}^{2+}$  ions both by diffusion towards the bulk and by having been trapped by EGTA. Thus, we do not think that the entry of residual  $\text{Ca}^{2+}$  ions during depolarization will be enough to develop tension transients as large as in normal Ringer's and lasting twice as long (Fig. 1). Therefore, we feel it more likely to be due to a release of  $\text{Ca}^{2+}$  from intracellular stores which may not be induced by  $\text{Ca}^{2+}$ .

In order to understand the mechanism(s) linking this calcium release to the action potential in Ca-free solution, we examined the membrane current and tension under voltage clamp conditions.

During exposure to Ca-free solution, by the time when the action potential duration was decreased (Fig. 2), the holding current (Fig. 3) was shifted to a steady value in the inward direction, while the slow inward current was transiently decreased (Fig. 3). Thus, the shortening effect of the decrease in slow inward current does not seem to have been sufficiently counteracted by the lengthening effect of the inward shift in background current. The subsequent lengthening of the action potential duration may be accounted for both by the increase in slow inward current and by its slower time course of inactivation (2–3 times slower than in control medium; Rougier et al. 1969), since in the meantime, the background current had not been changing (Fig. 3).

The secondary increase in amplitude and duration of the tension transients closely followed the increase in action potential duration (Figs. 1, 2). At this stage, the coupling mechanism might be attributed to a potential and/or a current dependent phenomenon.

Evidence has been brought that slow inward current in solutions lacking divalent cations, is carried by  $\text{Na}^+$  ions and is not influenced by TTX (Rougier et al. 1969; Garnier et al. 1969; Chesnais et al. 1975). In this work, we obtained evidence that the slow inward current in Ca-free solution behaves like a pure Na current (Fig. 4). This is in agreement with the Na-electrode like behaviour of the membrane during the plateau of the action potential in Ca-free solution when  $[\text{Na}^+]_0$  is varied (Garnier et al. 1969).

The slow inward sodium current-voltage and tension-voltage relations

(Fig. 5), obtained after 12 min in a 75 % Na (sucrose substitute) Ca-free solution containing  $10^{-6}$  mol/l TTX show that:

- the thresholds for tension and current development are similar;
- the tension-voltage relation exhibits a maximal value for a 40 mV depolarizing step at which the slow inward sodium current is maximum, and a minimal value for a 90 mV depolarizing step at which the current reverses in direction;
- for depolarizations larger than 90 mV, the curve relating tension to voltage raises again.

These results show that it is possible to record a "phasic" mechanical activity, which seems to correlate with the slow inward sodium current. At this stage, two arguments may be invoked to account for this result:

i) a lack of uniformity in potential within the preparation; ii) some  $\text{Ca}^{2+}$  ions entering the cells through the Na—Ca exchange if the cleft free calcium concentration has not been completely equilibrated with the bulk.

i) Due to the large increase in conductance when the slow inward sodium current is activated, the membrane resistance during the flow of the current would become comparable to the series resistance. The real voltage across the membranes of the preparation would then be larger than the command voltage and it would trigger amounts of voltage-dependent tension proportional to the increase in current. Thus, even if the slow inward sodium current per se were unable to trigger any tension development, the purely voltage-dependent increase in developed tension resulting from voltage "escape" would parallel the slow inward sodium current-voltage relation. In the preparation shown in Fig. 5, the amount of tension in response to a 40 mV command pulse was larger than when the command pulse was 100 mV in amplitude. To account for this tension response, the real potential during the 40 mV command pulse would be required to attain values over 100 mV. In the case of such a loss of voltage control, we might expect the depolarization of the membrane to be somewhat regenerative due to the increase in conductance, since this depolarization would tend to activate a larger conductance. This would show up in the current-voltage relation as a steepening of the negative slope region (Johnson and Liebermann 1971). It would also appear as a steep corresponding increase on the tension-voltage relation. This did not seem to be the case in our preparations (e.g. Fig. 5), where both the current and the tension were increasing smoothly over the potential range corresponding to the negative region of the current-voltage relation.

Furthermore, the time course of the deactivation of the current, when the command potential was returned to the HP after depolarization (see the tail current in Fig. 3), was fast and monoexponential (not shown). This indicates that no gross loss of voltage control occurred (De Hemptinne 1976). These arguments do not exclude, however that some of the tension component apparently correlated

with the slow inward sodium current, would be due to some imperfect voltage control, but they support the view that it should only be a minor part of it.

ii) The other hypothesis may explain why it was still possible to obtain some contraction when the slow inward sodium current was abolished by 3 mmol/l  $\text{Mn}^{2+}$  (Fig. 6B; Rougier et al. 1969). Indeed, the Na—Ca exchange would still be functional since 15 mmol/l  $\text{Mn}^{2+}$  are needed to block it completely. Furthermore, the mechanical tension elicited by depolarizations to beyond the reversal potential for the slow inward sodium current needs to be explained by a mechanism other than the release of  $\text{Ca}^{2+}$  induced by the entry of  $\text{Na}^+$  ions through the slow channel, since at these potentials,  $\text{Na}^+$  ions move outwards through the channel. In order to test the possibility of an inflow of  $\text{Ca}^{2+}$  ions through the Na—Ca exchange mechanism, we performed contracture experiments in calcium containing medium in the presence of Na—Ca exchange blockers: 15 mmol/l  $\text{Mn}^{2+}$  (Chapman and Ellis 1977; Ellis 1977; Horačková and Vassort 1979a; Coraboeuf et al. 1981) or 3 mmol/l  $\text{La}^{3+}$  (Horačková and Vassort 1979a) and in Ca-free solution without blockers.

The time course of the contractures was similar in Ringer-Mn, in Ringer-La (Fig. 8) and in Ca-free solution (Fig. 7). These results suggest that the tonic tension recorded in this latter medium was not due to calcium movement through the Na—Ca exchange mechanism, but might result from an internal release of  $\text{Ca}^{2+}$  ions which should be voltage-sensitive. This "voltage-dependent" tonic tension increased (Fig. 9) when the intracellular sodium concentration was elevated by repetitive voltage clamp stimulation in the presence of Veratrine ( $7.5 \times 10^{-6}$  g/ml) (Horačková and Vassort 1974).

This increase in voltage-dependent tension was regular throughout the experiment (25 min).

Such a behaviour has already been described for the release of insulin from isolated perfused islets of Langerhans of the rat (Donatsch et al. 1977). According to these authors, increased  $[\text{Na}^+]_i$  would be responsible for increased Ca release from internal sequestering structures, which would account for increased insulin release.

In summary, these results suggest the presence of a mechanism of intracellular calcium release which would be sodium-sensitive. Such a mechanism has been invoked in a variety of tissues (heart: Repke and Portius 1963; Palmer and Posey 1967; Carafoli et al. 1974; pancreatic cells: Lowe et al. 1976; skeletal muscle: Caillé et al. 1979; Potreau and Raymond 1982). Moreover, this mechanism could also be influenced by the membrane potential.

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