Contractility in Relation to Excitability in Voltage Clamped Crab Muscle Fibres: Evidence for Two Components of Tension

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Abstract. Electrical and mechanical activities were studied in voltage clamped crab muscle fibre. Two components of tension were described. The phasic component of tension is associated with the Ca inward current and the quantity of Ca ions entering the cell during the activation of this current. The tonic component of tension accounts for the tension elicited by low applied potentials which do not activate I_{Ca}, and for potentials higher than the reversal potential for Ca ions. When I_{Ca} is activated the tonic tension appears superimposed on the phasic tension and it accounts for the existence of the maintained tension at the end of long-lasting pulses. The tonic tension explains the existence of mechanical activity in passive fibres. The voltage and time dependences of the tonic tension were analysed and the mechanical threshold was described. The dependences of the tonic tension on external Na and Ca ions suggested a regulation by a Na—Ca exchange mechanism. It is concluded that calcium influx is a prerequisite for the development of tension. Two origins for the Ca influx were suggested: Ca enters the cell via the calcium current and via Ca influx linked to Na efflux.

Key words: Ionic currents — Crab muscle fibre — Na Ca exchange — Phasic tension — Tonic tension

Introduction

Electrical activity in crustacean muscle fibres is due to the development of a calcium inward current (I_{Ca}) which can be recorded under voltage-clamp conditions (Hagiwara et al. 1969; Keynes et al. 1973). This electrical response is generally graded because of the simultaneous activation of I_{ca} and of a potassium outward current, I_{K_1} (Mounier and Vassort 1975 a, b; Henček and Zachar 1977). Some crab muscle fibres exhibit only passive electrical responses. Whatever the electrical activity is, passive or active, crab muscle fibres develop mechanical activity as already shown by Atwood (1963) and Atwood and Dorai Raj (1964).

Whether the calcium influx is essential or not for the contraction of muscle fibres is not yet clear as illustrated by the recent letters between Caputo and Dipolo

(1980) and Edwards (1980). Two hypotheses exist and have been described as inconsistent. The first one results from the relationship between membrane potential and tension established in barnacle muscle fibres (Edwards et al. 1964; Dudel et al. 1968; Hagiwara et al. 1968; Ashley and Ridgway 1970; Caputo and Dipolo 1978). The shape of the relationship is sigmoid i. e. the amplitude of tension increases as the depolarization increases and reaches a plateau for depolarizations higher than the equilibrium potential for calcium ions. This means that tension can be recorded in the absence of I_{Ca}. The second hypothesis has its origin in the result of Suarez-Kurtz et al. (1972). These authors considered that a change in membrane Ca conductance is an essential step in excitation contraction coupling in crayfish fibres. This has been reinforced by experiments of Atwater et al. (1974): the calcium current is necessary for the development of tension.

The variability of the electrical activity in crab muscle fibres related to the variation of I_{Ca} an I_{K_1} amplitudes led us to an attempt to clarify whether calcium influx is required for the generation of mechanical activity. We used simultaneous recording of tension and membrane currents under voltage-clamp conditions by means of the double sucrose gap technique. Two components of tension which reconcile the two mentioned hypotheses have been described.

Material and Methods

Muscle fibres: Experiments were performed at 18°C in single fibres of the extensor muscle isolated from the meropodite of the walking legs of the crab *Carcinus maenas*. Their diameters varied from 150 to 200 μm. The animals used for experiments were always in intermoult state and the same fibres from the same muscle were used. Despite these standard conditions we observed that most of the fibres obtained in winter were electrically passive while graded responses were recorded during the other seasons. Such a discrepancy has not been further analysed. Yet, Atwood et al. (1965) reported similar observations and suggested that hormonal or other long-term changes can determine to some extent the membrane properties of the fibres and cause a conversion from one kind to another.

Sucrose-gap arrangement and records: The preparations were put in a sucrose-gap apparatus which allows both electrical and mechanical recordings. This method follows that of Vassort and Rougier (1972) and has been adapted to crab muscle fibres. A more detailed description and the reliability of the voltage-clamp method were reported in previous papers (Mounier and Vassort 1975 a, b, 1979). The mechanical tension, T, was measured in the test compartment with a transducer element (series 8101 from Pixie) lengthened by a thin lever (3 cm long); with amplifiers and filters used its characteristics were: resonance frequency 90 Hz, sensitivity 150 mV/10 mg, linear response up to more than 350 mg. The lever was put tangentially in the center of the artificial node (100 μm long) and a preload was applied, in order to stretch the fibre, before recording the tensions elicited by different pulses. The force (R) measured by means of the transducer is the resulting force of the linear force (F) developed by the fibre on each side of the transducer needle, the needle being in the centre of the test compartment. Then $R = 2F \cos \alpha$ with α = angle formed by the fibre axis and the displacement plane of the needle which is 60°.

Expression of the results and nomenclature: The following nomenclature was used. V is the variation of the membrane potential from the resting potential, E_R, taken as zero. Positive values of V are depolarizations. I is the membrane current, positive if outward. It has been corrected, using an analog system, for the leak and most of the capacitive currents. The correction for the leak current has been made for small applied depolarizations and hyperpolarizations, assuming a linear voltage dependence for larger depolarizations. This approximation is valid for depolarizations up to 65 mV while for larger depolarizations a slight outward rectification is apparent (Mounier 1975). The correction for the capacitive current does not modify our results since the calcium peak occurs after 5 ms or more while the capacitive current declines with a time constant of 1 to 2 msec. I_i represents the inward component of the initial composite current described as the result of simultaneous activation, with similar kinetics in opposite directions, of the calcium inward current, I_{Ca}, and the fast potassium outward current, I_{K_1} (Mounier and Vassort 1975b; Henček and Zachar 1977). Thus, in normal solution (ASW, see below), I_i reflects the Ca current, and the applied potential inducing $I_i = 0$ is the reversal potential, V_{rev}, of the inward component of the composite initial current. V_{rev} generally ranges from +75 to +80 mV (Mounier and Vassort 1975a). In presence of an inhibitor for I_{K_1} (TEA), I_{Ca} can be recorded alone and the measured reversal potential is the reversal potential for Ca ions, V_{Ca}. The value of V_{Ca} is higher than that for V_{rev} (Mounier and Vassort 1975a).

Bathing solutions: The test gap was bathed in standard solution, artificial sea-water, ASW, which had the following composition (mmol/l): NaCl, 513; KCl, 12.7; $CaCl_2$, 11.8; $MgCl_2$, 23.6; $NaHCO_3$, 2.6; pH, 7.8. Other test solutions were derived from ASW. Ca-rich solution was obtained by increasing the $CaCl_2$ concentration ($CaCl_2 \times 5$) and decreasing the NaCl content maintaining both the osmotic pressure and the chloride concentration constant. This induced a 14 per cent decrease in $[Na]_o$. Ca-free solution or Ca 0 solution was obtained by reducing the $CaCl_2$ concentration (sucrose added). Sometimes, EGTA (ethylene glycol-bis-(aminoethylether) N-N'-tetraacetic acid) (10^{-4} mol/l) was added to Ca 0 solution. Manganese solution or Mn solution was prepared by adding $MnCl_2$ (5 mmol/l) to the ASW solution. It was used to inhibit the calcium current. TEA (TEA-Cl from BDH, 50 mmol/l) was used to inhibit the K conductances. When reducing or suppreassing Na ions in the external solution, NaCl was substituted by LiCl. In Na-free medium (NaCl 0, LiCl) a strong contracture develops and then relaxes. The experiment designed to record tension in this medium was carried out when the fibre was in the state of relaxation (Fig. 6). In other experiments, the development of tonic tension in Na-deficient conditions was controlled (Fig. 11); low-Na solutions (NaCl 1/3, LiCl 2/3) were used for this purpose since under such conditions contracture does not develop. In Na-free and low-Na media a slight membrane hyperpolarization occurs (about 5 mV) and this was also taken as the reference potential or holding potential from which the applied membrane polarizations are given. The pH of all solutions was always maintained at 7.8 by Tris-HCl. Exchange of solutions is quick since the new medium reaches the test gap within 0.25 sec.

Results

Reliability of tension recording

It is well known that crab muscle fibres exhibit diverse forms of electrical responsiveness associated with various contractile responses (Atwood et al. 1965). Similar diversity has been found in our experimental conditions. This is illustrated in Figure 1.

On gradedly responding fibres (Fig. 1A) increasing the amplitude of the

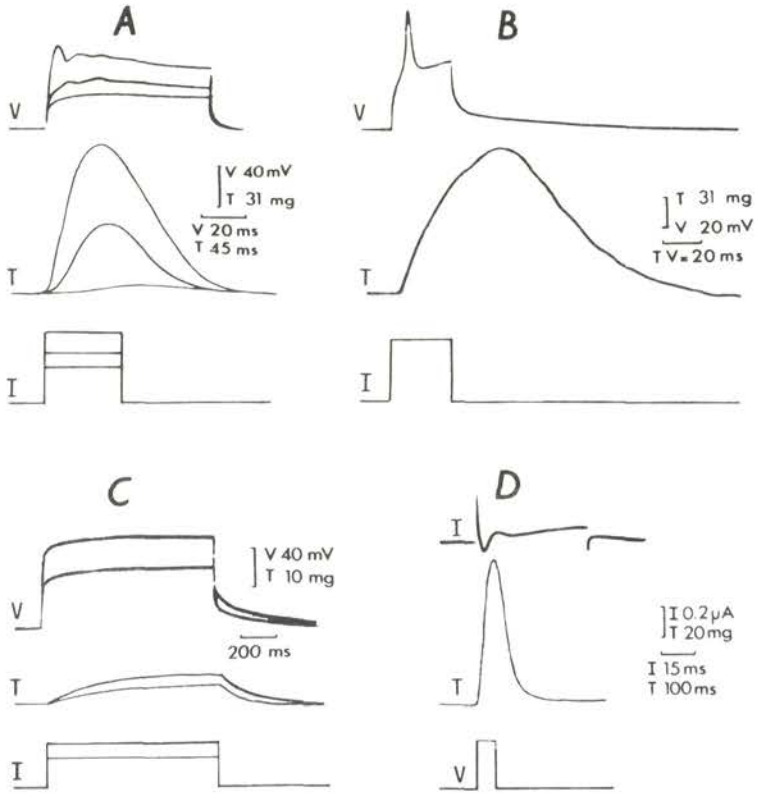

Fig. 1. Records of electrical and mechanical activities. A, B, C: records of membrane potential changes (V) and tension (T) under current-clamp (I) conditions, in a gradedly responding fibre (A), an all-or-none spiking fibre (B) and in a passive fibre (C). Same scale for each current step I, not calibrated in μA. D: record of ionic current (I) and tension (T) under voltage-clamp conditions for 70 mV, 60 ms depolarizing step (V).

applied current (75 ms duration) induces a gradual increase in both the potential response and the amplitude of the tension. The tension is phasic and the threshold for mechanical activity appears when the membrane is depolarized by about 25 mV. When the action potential attains a maximum, the tension is maximum as well; for large applied currents no further increase in tension is observed. The characteristics of the maximum tension are as follows: the amplitude reaches about 100 to 150 mg and the time to peak ranges from 50 ms to 70 ms. The latter is in good agreement with the value reported by Atwood el al. (1965) for gradedly responding fibre stimulated by a microelectrode and using a RCA 5734 transducer tube to record tension. The former is in the low part of the range reported by the same authors: 150 to 500 mg.

In all-or-none spiking fibres (Fig. 1B) the associated tension is a twitch as reported by Atwood et al. (1965). In passively responding fibres no action potential can be recorded, whatever the amplitude of the applied current is (Fig. 1C). The simultaneously recorded tensions are tonic, i. e. a plateau of tension is maintained as long as the membrane is depolarized (1s) and the kinetics are slow. The amplitude of the maximum tension is lower than in graded fibres. Atwood et al. (1965) reported identical observations.

The similarity of our results with those of Atwood et al. (1965) is a convincing argument for the reliability of our method to record tension, despite the fact that the devices used by us to record electrical and mechanical activities are different. Under voltage-clamp conditions (Fig. 1D) a 70 mV depolarization has been applied in order to reproduce in amplitude the membrane depolarization at the peak of a graded action potencial. Under these conditions both ionic currents and tension have been recorded. Although ionic currents will be explained in the following paragraph (Fig. 3), it must be put forward at this place that the characteristics of the tension are close to those associated with the maximum action potential under current-clamp conditions. The time to peak varies from 55 to 65 ms and the amplitude equals 90 mg. This is an important result since it rules out the possibility of an artefact, especially of the phasic tension, since under voltage-clamp conditions the fibre is stimulated in the test gap and under current-clamp conditions in the lateral gap.

Moreover, under voltage-clamp conditions the largest amplitudes of tension reach 200 to 250 mg, i. e. 1 to 1.5 kg/cm^2 with a fibre diameter of 200 µm. This value of maximum tension agrees with those proposed by Atwater et al. (1974) and Caputo and Dipolo (1978) in another crustacean muscle fibre (barnacle) under voltage-clamp conditions using internal electrodes.

Finally, the possibility that inward currents in the test node lead to contraction in neighbouring parts of the fibre whose potential is not under voltage control has been ruled out by the following experiment (Fig. 2). The tension elicited by

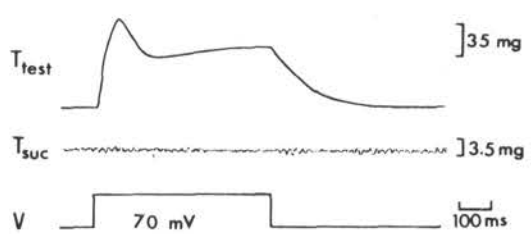

Fig. 2. Records of tension (T) for 70 mV, 550 ms depolarizing step (V). Tension has been simultaneously recorded in the test gap (T_{test}) and in the sucrose gap (T_{suc}). Fibre n° 203.

a 70 mV, 550 ms depolarization has been simultaneously recorded in both the test gap and one sucrose gap. The results show that a typical tension, whose characteristics will be described later (Fig. 3) develops in the test gap. On the contrary no tension can be recorded in the sucrose gap. Similar experiment has been done by recording in the other gap. Also in that case, no tension developed.

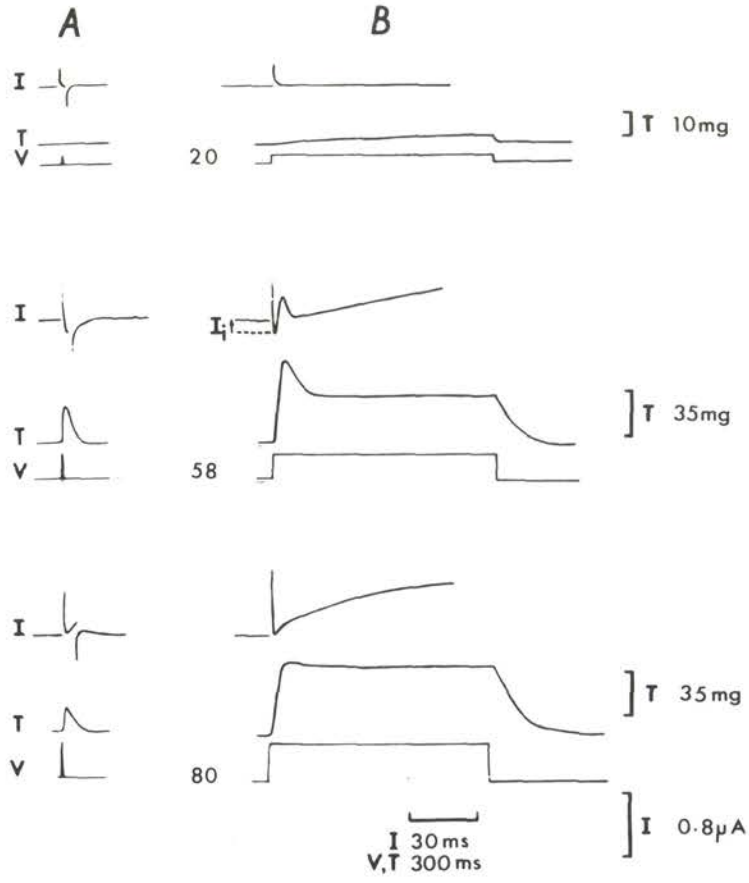

Fig. 3. Records in ASW solution of currents I and tension T under voltage-clamp conditions for two durations of applied pulses V (A: 5 ms depolarizations, B: 1000 ms depolarizations). The amplitude of V is indicated on the records: 20,58 and 80 mV. I_i indicates the inward component of the initial current (see Methods). The leak current and a part of the capacitive current have been corrected using an analog system. Fibre n° 211.

Records of currents and associated tensions elicited by membrane depolarizations of different durations

Short-lasting depolarizing steps: The ionic currents and phasic mechanical responses due to 5 ms depolarizing steps in ASW solution are illustrated in Fig. 3(A). This duration allows the inward component (I_i) of the initial current to be maximally activated at all potentials and is sufficient for the electrical signal to spread inwards into the center of the fibre (Dudel at al. 1968). For a 20 mV depolarizing step neither I_i nor contraction are elicited. For higher potentials, as illustrated for 58 mV depolarization, the inward current is activated and a transient tension develops. For a higher depolarization (80 mV) only the slow outward current I_{K2} (see Mounier and Vassort 1975a) is activated, well characterized by the outward tail current which develops when the membrane potential returns to the resting potential. A smaller tension is recorded.

Long-lasting depolarizing steps: The ionic currents and mechanical responses, recorded in the same fibre, up to 1000 ms depolarizing steps of varied amplitude are illustrated in Fig. 3 (B). Some new points must be put forward: i) a tension maintained as long as the membrane is depolarized appears at the 20 mV depolarization; ii) at the 58 mV depolarization, the tension exhibits a complex time course with a phasic peak followed by a maintained steady-state level higher than the one observed at the 20 mV pulse; iii) this complex time course is not so obvious when 80 mV depolarization is applied; the tension reaches a large amplitude with a small initial peak and is maintained.

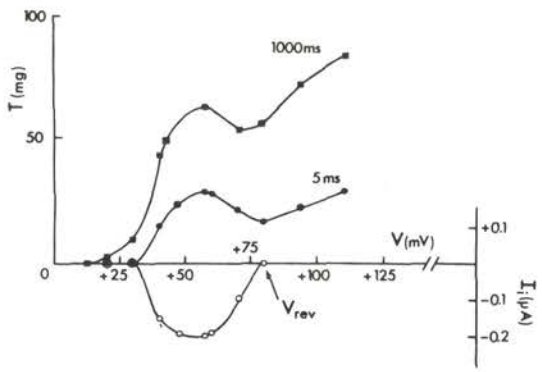

Fig. 4. Current voltage and tension-voltage relationships. Tension amplitudes were measured at the peak for 5 ms (filled circles) and 1000 ms (filled squares) depolarizations. Open circles represent peak values of the inward component I_i of the initial current. The same fibre n° 211 as in Fig. 3.

Current — tension — voltage relationships: In Fig. 4, the peak amplitude of contraction elicited by clamp pulses of two different durations (5 and 1000 ms), and the amplitude of the I_i current measured at its peak, were plotted against the membrane potential during the steps. The mechanical threshold coincides with the inward current threshold ($V = +31$ mV) for the 5 ms pulse, while it is lower when the duration of the pulse is increased. For depolarizations higher than the I_i threshold, I_i and tension increase rapidly up to a 55 mV applied step. In the range of 55—85 mV the inward current decreases and becomes nought at the reversal potential, V_{rev} (+83 mV); the contraction simultaneously decreases, too. At larger depolarizations, the tension increases although I_i is suppressed or outward. The amplitude of tension increases with the duration of clamping voltage.

The contractile response seems to be composed of two components: a component related to the inward current and another one which can explain small tensions observed at small depolarizations and large tensions obtained at large depolarizations as well as the tension recorded at the end of long-lasting pulses.

Ca inward current and its relation to the phasic component of tension

To determine the relation between the phasic component of tension and the inward current, experiments have been performed under conditions which induce I_i change. Moreover, to establish the relation between the calcium current and the phasic tension, some experiments have been performed in TEA solution in order to suppress the contamination of I_{Ca} by I_{K1} and I_{K2} currents.

Modifications of the inward component of the composite initial current: effect on the phasic tension

To minimize the influence of the component of tension which is more important at the end of long duration pulses (described later as "tonic" tension), we applied short duration pulses. In Ca-rich solution ($[Ca]_0 \times 5$: "Ca 5"), the inward component I_i of the initial composite current and the tension elicited by 55 mV, 15 ms depolarization are enhanced. In calcium free solution ($[Ca]_0 = 0$: "Ca 0"; EGTA added or not) or in Mn (5 mmol/l) solution the inward current is suppressed and the tension disappears as soon as the solution reaches the cell (Fig. 5).

Relation between the calcium current and the phasic tension: To avoid any contamination of I_{Ca} by I_{K1} and I_{K2} the following experiments were performed in TEA solution. Two types of experiments were carried out. One to establish the voltage dependence of I_{Ca} and of the phasic tension as well as to determine the amount of calcium ions flowing into the cell during depolarization. Another one to

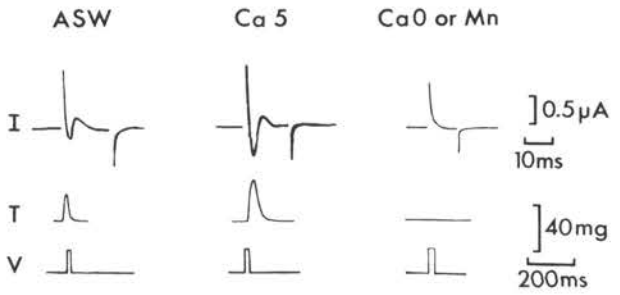

Fig. 5. Effect of various extracellular Ca concentrations ($[Ca]_o \times 5$: "Ca 5" and Ca free: "Ca 0") and of Mn ions (5 mmol/l) on the initial inward current (I) and the phasic tension (T) elicited by 55 mV, 15 ms depolarization (V). In the "Ca 5" solution NaCl concentration has been reduced by 14 per cent compared with standard solution. Fibre n° 2303.

determine the voltage dependence of the calcium current inactivation process using either I_{Ca} or phasic tension measurements.

a — To evaluate the amount of calcium ions flowing into the cell during Ca current activation, a 100 ms depolarization was chosen. For this duration the tonic component of tension is not negligible especially at high depolarizations. In order to suppress it, NaCl was replaced by LiCl (see later). This condition does not modify the calcium current.

The amplitude of the phasic tension follows that of the calcium current as shown in Fig. 6 (A) for three different potential levels. This is confirmed by the relationships between potential, peak amplitude of the calcium current and amplitude of the tension (Fig. 6B). The I-V curve is a typical one (see Mounier and Vassort 1975a, their Fig. 6). The T-V curve has the same shape as the I-V one; both have a similar threshold (24 mV depolarization in Fig. 6A), they increase as the applied potential increases until 42 mV depolarization and then, both relationships decrease as shown for the 90 mV pulse (part A). Finally, I_{Ca} and T are suppressed at the reversal potential for Ca ions, V_{Ca}, equal to +116 mV. For higher pulses, I_{Ca} is outward while tension remains nought.

The amplitude of tension was also correlated with the quantity of Ca ions entering the cell Q_{Ca}. This quantity is represented by the total amount of the Ca inward current, during the pulse (Q_1) and of the tail current when clamping back to E_R (Q_2) (see Fig. 6B, inset):

$$Q_{Ca} = \int I_{Ca}\,dt = Q_1 + Q_2,$$

where $\int I_{Ca}\,dt$ is the integral of I_{Ca}.
The area measurements of currents were performed graphically. The relationship

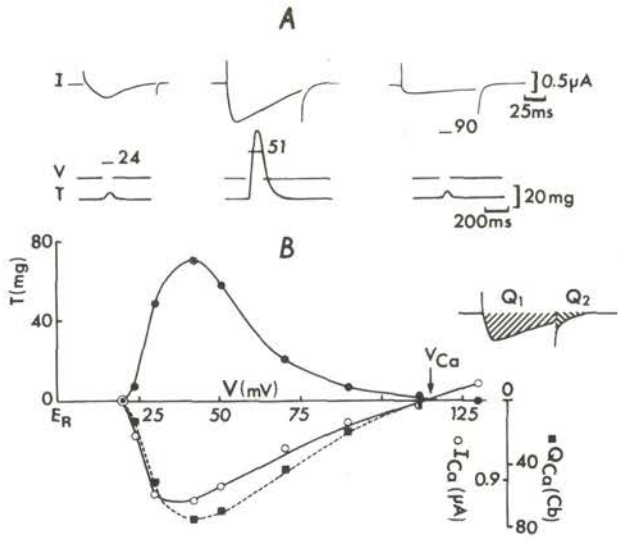

Fig. 6. Relation between the inward calcium current and phasic tension established in TEA (50 mmol/l) Na free solution. (All results from fibre n° 181). A: Records of inward calcium current I and phasic tension T elicited by three depolarizations V (24, 51, 90 mV). B: Relationships between the applied depolarizations V, the inward calcium current I_{Ca} (open circles) and the phasic tension (filled circles). The dashed curve (filled squares) represents the dependence of Q_{Ca} on the applied potentials. $Q_{Ca} = Q_1 + Q_2$, quantity of Ca ions entering the cell during depolarization as indicated on the inset. V_{Ca}: reversal potential for I_{Ca}.

Q_{Ca} vs. the imposed potential parallels well with the T-V curve (Fig. 6B). Similar results were obtained on three other fibres.

b — Pre-depolarizations of different magnitude were applied in order to determine the voltage dependence of the Ca current inactivation process expressed as the f_∞(or I/I_{max}) curve. Tensions were simultaneously recorded to establisch the voltage dependence of T/T_{max}. Pre-depolarizations of 300 ms were used since this duration allows both total inactivation of I_{Ca} and relaxation of the phasic tension to a steady level due to the tonic component of tension. Fig. 7(A) shows a series of records from that experiment performed in TEA solution using 60 mV, 50 ms test-depolarization. The availability curve (f_∞ or I/I_{max}) vs. pre-depolarization, V, was drawn according to the following equation (Fig. 7B):

$$I/I_{max} = f_\infty = 1/(1 + \exp[(V - V_f)/k]),$$

where I is the amplitude of calcium current elicited by the test-pulse after different pre-depolarizations and measured from the holding potential, I_{max} is the maximal

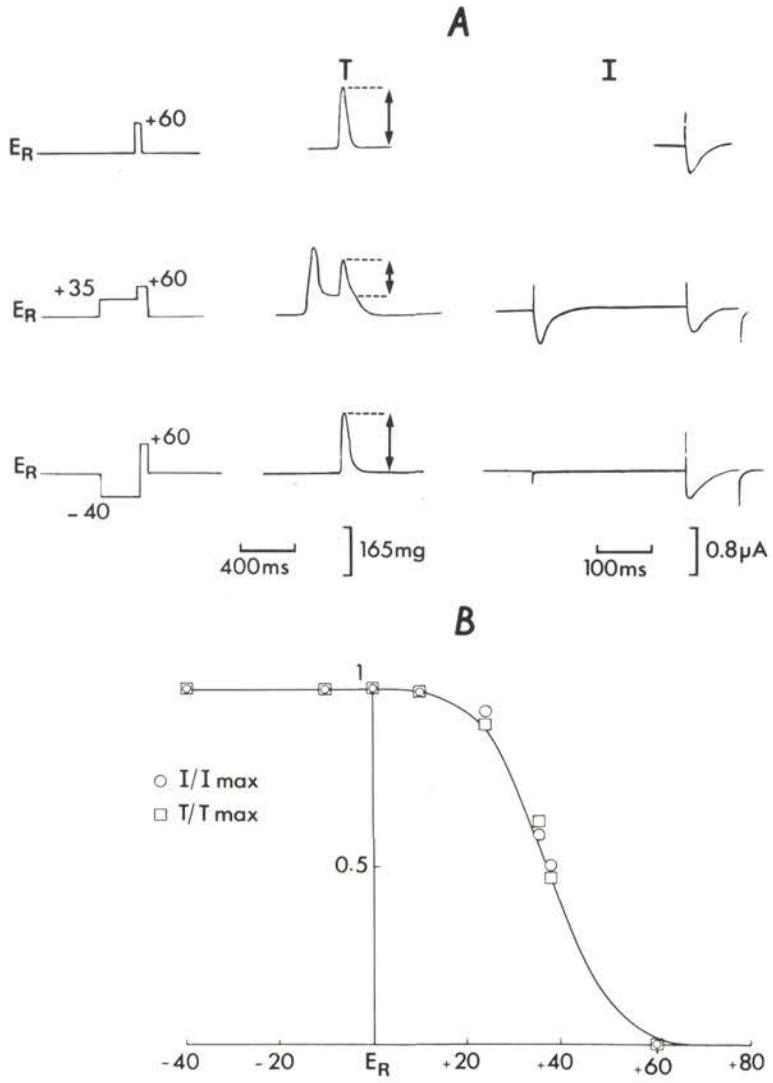

Fig. 7. Dependence of the inward calcium current I(A right) and phasic tension T (A centre) on the membrane polarization (A left). In A records for 60 mV test pulse are illustrated, without a conditioning pulse, with 35 mV pre-depolarization and 40 mV pre-hyperpolarization. Arrows indicate the tension amplitude elicited by the test pulse. Part B represents the inactivation curves for I. Open circles represent values in ordinate of the ratio I/I_{max} for different pre-pulses in abscissa (values are displacements from E_R). Open squares represent values of the ratio T/T_{max}. (See text for explanations of I/I_{max} and T/T_{max}). Fibre n° 308.

value of I, V_f is the half-availability potential (+36 mV in the illustrated fibre), k is the slope factor (=7 mV). This availability curve is typical since pre-depolarizations larger than 15 mV decrease the amplitude of I_{Ca} as shown in Fig. 7A for the 35 mV pre-pulse. Calcium current is unavailable for pre-pulses higher than 60 mV and finally, prehyperpolarizations induce no modification of I_{Ca} as illustrated for 40 mV pre-hyperpolarization in Fig. 7A. Values of T/T_{max}, with T, the phasic tension elicited by the test-pulse and measured as indicated by arrows in Fig. 7A and T_{max}, the maximal value of T, are given on the calcium current availability curve (Fig. 7B). These values fit the curve, implying identical voltage dependence of I/I_{max} and T/T_{max}. Similar results were obtained in 6 other fibres. This result does not exclude the idea that Ca-inactivation might depend on Ca entry. Since this paper deals only with the excitation-contraction coupling, more experiments will not be discussed here.

Analysis of the tonic component of tension

The maintained component of tension which appears for long-lasting depolarizations has been called the tonic component of tension. We studied it under conditions which suppress influences of I_{Ca} and phasic tension, i. e. at the end of long-lasting pulses when I_{Ca} is inactivated and the correlated phasic tension relaxed. We also found an easier way to record this tension alone by performing experiments in passive fibres.

Evidence for a tonic component of tension in passive fibres: Figures 8 and 9 (A) show ionic currents and mechanical activity in passive fibres, i. e. those which only

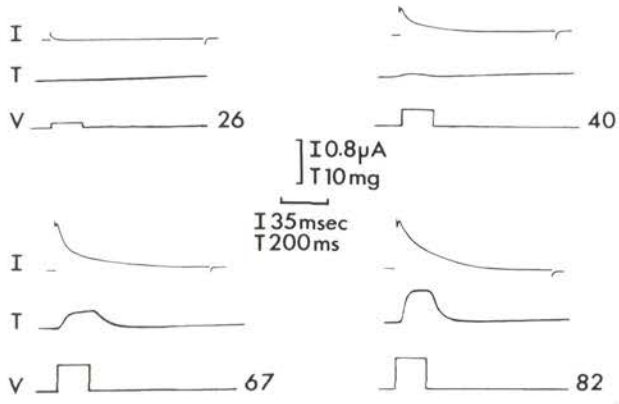

Fig. 8. Records of the ionic currents (I) and tonic tension (T) for different depolarizing steps V of 150 ms duration applied on a passive fibre n° 192 (amplitudes of V are given on the records).

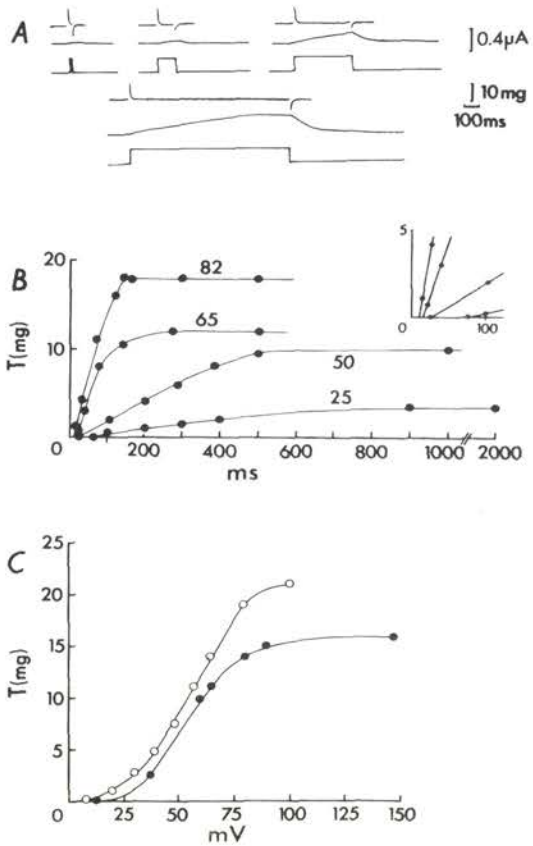

Fig. 9. Time and voltage dependences of the tonic tension in ASW solution. A: Records of ionic currents (top) and tonic tension (centre) elicited by 50 mV depolarization of different durations (bottom). Note that neither I_{Ca} nor I_{K1} exist (fibre n° 6578). B: Time dependence of the tonic tension: amplitudes of the tonic tensions (ordinate) elicited by pulses of different durations (abscissa). The amplitude of each pulse is given on the curves. The inset corresponds to an amplification of the initial part of the different curves (fibre n° 6578). C: Voltage dependence of the tonic tension. Amplitude of tension are taken at their maximal steady-state values, at the end of 1000 ms pulses in a passive fibre (filled circles) and in an excitable fibre (open circles) (fibre n° 6278 and n° 7278).

exhibit electrotonic potentials under current-clamp conditions as described in Fig. 1B.

For the illustrated depolarizing steps of 150 ms duration in Fig. 8, a fast outward current is activated. This current, previously described as the I_{K1} current (Mounier and Vassort 1975b) has an activation threshold about 35—40 mV and is quickly inactivated. No inward current can be detected but a tension develops slowly and is maintained as long as the membrane is depolarized. In Fig. 9 (A), the

fibre does not exhibit I_{K1} current. Current record elicited by 50 mV depolarization shows the capacitive current only since the leak current has been corrected by an analog system (see Methods). Similar tensions as in Fig. 8 are recorded. About 35 per cent of passive fibres do not show I_{K1} activation (15 experiments).

Time and voltage dependences of the tonic tension: The amplitude of the tonic tension recorded in passive fibres increases up to a maximum value with the duration of the depolarization. This is illustrated in Fig. 9 (A). Results obtained in one fibre with different pulses of different durations are summarized in the Fig. 9B. It clearly shows that the higher the pulse the shorter the duration required to reach the maximum tension; it varies from 120 ms and 82 mV depolarization to 900 ms and 25 mV depolarization. Moreover, the duration required to elicit the smallest tension is inversely proportional to the amplitude of depolarization (inset, Fig. 9B). Similar complete results were obtained in two other fibres.

The voltage dependence of the tonic tension was studied with pulses which were long enough for the tension to develop up to the maximum steady state at all levels of potentials. This was carried out both in passive and excitable fibres (Fig. 9C) with 1000 ms pulses. The amplitude of tension increases as the amplitude of the depolarization is increased up to 100 mV. At higher depolarizations the tensions remain saturated at the maximum. Moreover, there is always a mechanical threshold close to 20—25 mV. The two relationships established for the tonic tension both in passive and excitable fibres are similar. The same results were obtained in five other passive fibres and in six other excitable fibres at the end of long lasting pulses.

Dependence of the tonic tension on extracellular ionic concentration: Ca ions: Experiments were performed in a Ca-free medium "Ca 0" (with EGTA) (Fig. 10A). In a passive fibre the tonic tension elicited by a 57.5 mV, 500 ms depolarization is completely abolished after 30 s in this medium. The tension is suppressed whatever the amplitude of the depolarizing step is, with a good reversibility. In manganese solution (Mn^{++} 5 mmol/l) the tension recorded in a passive fibre progressively decreases and the time to maximum tension increases (Fig. 10B). After 5 minutes the effect of Mn ions is fully developed; the tension is small but does not disappear. With a long-lasting pulse (7 s) applied to an excitable fibre (Fig. 10B) a small tension remains after Mn treatment while the phasic tension and the Ca current are suppressed (as already described in Fig. 5). Manganese in concentration higher than 5 mmol/l (10—20 mmol/l) acts quicker and entirely abolishes the tension. The Mn ions may inhibit Ca influx other than I_{Ca} and thus could interact with I_{Ca} at a place other than the calcium channel. However

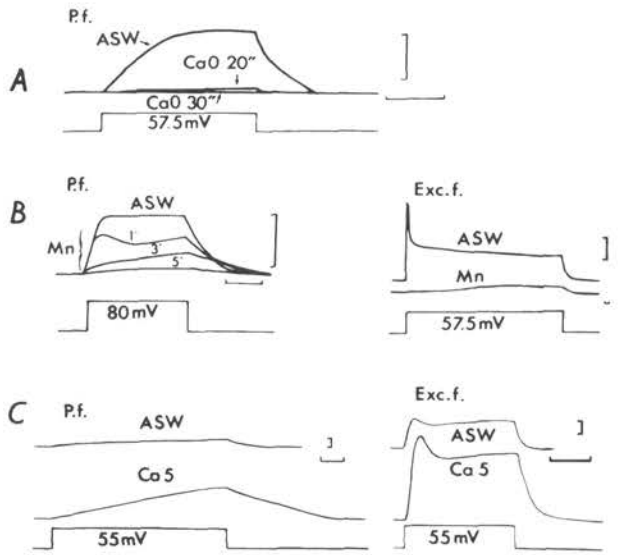

Fig. 10. Dependence of the tonic tension on extracellular calcium concentration. Records of the tonic tension for long duration pulses applied on excitable fibres (Exc. f.) or on passive fibres (P. f.). Tensions upper traces, pulses lower traces (amplitudes indicated). A: Tension elicited by 57.5 mV, 500 ms depolarizing step in ASW solution and in Ca free solution ("CaO") (EGTA, 10^{-5} mol/l added). Note the suppression of the tonic tension. Record from a passive fibre. B: Effect of Mn ions (5 mmol/l) on the tonic tension in passive (left traces) and excitable (right traces) fibres. C: Increase in the tonic tension elicited by 55 mV depolarization in passive (left records) and excitable (right records) fibres in Ca rich solution. "Ca 5" indicates $[Ca]_o \times 5$. In A and B (left) superimposed records traced from originals. The horizontal scales correspond to 200 ms and vertical scales to 10 mg.

the latter and as a consequence the phasic tension are more sensitive to Mn than the tonic tension.

In a Ca-rich medium ($[Ca]_0 \times 5 =$ "Ca 5"), the tonic tension of a passive fibre increases by a factor 3.5 to 5 (4 for the illustrated fibre) (Fig. 10C). This can be also seen in excitable cells at the end of a 55 mV, 550 ms depolarization. The enhancement of the phasic tension which was previously described (Fig. 5) is obvious again.

Na ions: Fig. 11 shows the effect of low-sodium solution (NaCl 1/3, LiCl 2/3) in an excitable fibre. In order to suppress the influence of the phasic tension a high amplitude pulse of 100 mV was used and to reach the steady-state amplitude of the tonic tension (see Fig. 9B) the duration of the pulse was chosen to be equal to 400 ms. Under these conditions, the tonic tension decreases to 45 per cent of the control tension in ASW (in Fig. 11A, compare record in ASW and record *b*) and is

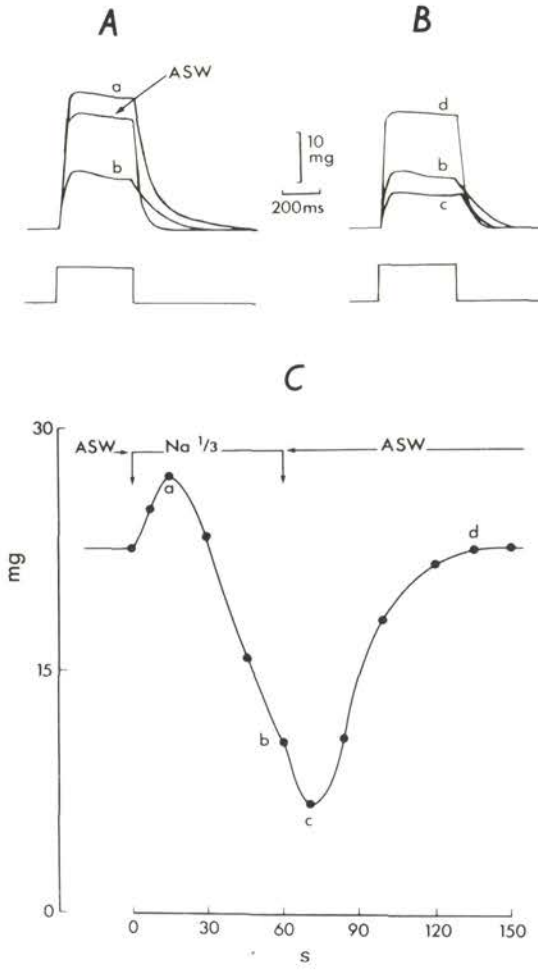

Fig. 11. Dependence of tonic tension on extracellular sodium concentration. A: Evolution of the tonic tension elicited by 100 mV depolarization in low-Na solution ($[Na]_o = 1/3$, $[Li]_o = 2/3$). The record *a*, after 15 s of the Na 1/3 solution, shows an increase in tonic tension compared with the record in ASW. Record *b* shows a decrease in the tension after 1 minute. B: Evolution of the tonic tension elicited by 100 mV depolarization on return to ASW. Record *b* is the same as in part A. Record *c* shows a decrease in tension after 15 s in ASW. Record *d* shows the total recovery of tension amplitude. C: Graph illustrating the evolution of the amplitude of the tonic tension (ordinate) plotted against the time (abscissa), upon substitution of ASW by Na-low solution (Na 1/3) and when returning to ASW. A, B and C are the same fibre n° 1505. For A and B: superimposed records traced from originals.

maintained at this level as long as the low sodium solution is not washed out. Depending on the fibre, the amplitude of the tonic tension reaches 50 to 30 per cent of the control level. Two experiments have also been carried out using

a Na-poor solution with choline chloride as a substitute for NaCl. In that case, the amplitude of the tonic tension was steady at 68 and 42.5 per cent of the tension in ASW.

A more accurate study immediately after the application of the Na-poor medium (NaCl 1/3, LiCl 2/3) shows a transient increase of the tension reaching maximum (17 per cent of the control value) after 15—20 s (Fig. 11A, record *a*). The ampitude of this maximum transient enhancement varies, from fibre to fibre, from 10 to 17 per cent of the control value (4 different fibres). Then the tension decreases and after 1 minute it reaches the low amplitude reported above, i. e. 45 per cent of the control tension in ASW (Fig. 11A, record *b*). The relaxation phase is prolonged. On return to ASW the tonic tension further decreases (Fig. 11B, record *c*) before returning to the control level (Fig. 10B, record *d*) in 75s. These results are summarized in Fig. 11C.

Discussion

The reported results show the relations between membrane potential, calcium current and tension in crab muscle fibre. The mechanical activity is characterized by a phasic and a tonic component of tension.

Two components of tension

The phasic component: The experiments for proving the reliability of our method to record tension were reported in the Results (Fig. 1 and Fig. 2). They show that the phasic tension is neither an artefact, nor a hump on the tonic tension since it can be elicited alone by short-duration pulses which induce I_{Ca} activation (Fig. 3A and Fig. 5), and in physiological saline which inhibit the tonic component (Fig. 6).

Phasic tension is dependent on the extracellular calcium concentration since it is increased in Ca-rich solution and suppressed in Ca-free medium or in the presence of Mn ions (Fig. 5). Besides ionic sensitivity, the most important result to support the relation between the phasic tension and the calcium current is their identical dependence on membrane potential. Indeed, the voltage dependence of the phasic tension follows that of either I_{Ca} or the quantity of calcium ions entering the cell during I_{Ca} activation, Q_{Ca} (Fig. 6B). Moreover the voltage dependence of the Ca current inactivation process shows similar relationships whether determined from I_{Ca} or peak phasic tension (Fig. 7). Such a direct relation between I_{Ca} and tension or Q_{Ca} and tension has been described in barnacle muscle fibres by Hidalgo et al. (1979). We have got evidence that phasic tension is specifically linked to Ca current (I_{Ca}) rather than to the inward current (I_i). Figure 3B, shows that for an 80 mV depolarizing step, i.e. positive to the reversal potential (V_{rev}) for I_i, a small phasic peak is still recorded. I_i is the sum of I_{Ca} and I_{K1} and since I_{K1} is outward we can expect that a calcium influx takes place at potentials positive to the apparent V_{rev}

estimated in ASW. This is indeed the case since the reversal potential for Ca ions measured under TEA conditions (for further explanations see Material and Methods) is more positive than V_{rev}. Under these conditions phasic tension is nought at V_{Ca}. The very low value of V_{Ca} can be due, as reported by Mounier and Vassort (1975a), to local accumulation of calcium ions evaluated as 1—2 mmol/l in the diad juntions by Zacharová and Zachar (1967).

The tonic component: We described it in passive fibres and in excitable cells at the end of long lasting pulses and a tonic component of tension, maintained as long as the pulse is applied. We consider that it is the same tension in both cases since their voltage and ionic dependences are similar. The amplitude and time course of this tonic tension (Fig. 9) is determined by the amplitude and duration of membrane depolarization.

The explanation of the tonic tension component may be looked for in its ionic dependence on Ca and Na ions reported in the results (Figs. 10 and 11). Previous reports on crab and barnacle muscle fibres suggested the existence of a Na—Ca exchange mechanism (Baker 1972; Vaughan Jones 1977; Russell and Blaustein 1974). Recently Mullins (1977), Horackova and Vassort (1979) showed that the direction and the amplitude of the electrogenic Na—Ca exchange is voltage dependent. The Na—Ca exchange was found to reverse (Ca influx — Na efflux) and the amplitude of the calcium influx to increase with the depolarization. Thus, we can assume that the tonic component of tension is due to Ca influx related to Na—Ca exchange activated by membrane depolarization. In our experimental conditions, no current due to Na—Ca exchange is recorded. However, this does not exclude the possibility of its electrogenicity. Indeed, assuming a ratio of Ca/xNa with $x = 3$ (Horackova and Vassort 1979), the resulting current would be outward and it would be included in the other outward currents (the leak current and the two K currents, I_{K1} and I_{K2}).

Another explanation for the origin of the tonic tension which has to be considered is the mechanism of sodium induced Ca release as described by Caillé, Ildefonse and Rougier (1978) for the activation of contraction of frog twitch muscle fibre. However, our results suggest rather tha Na—Ca exchange mechanism and this for three reasons: 1) in Ca-free solution the tonic tension disappears as soon as the solution reaches the fibre, 2) in a Na-poor medium, tension increases transiently while it transiently decreases on return to ASW. This could be explained in the same way as in the heart muscle (Horackova and Vassort 1979). Following a decrease in the external sodium concentration, Na efflux is transiently stimulated and so is the associated Ca influx; following an increase in the external sodium concentration (return to ASW), Na efflux is transiently depressed inducing a decrease in the associated Ca influx; 3) slight hyperpolarization in the Na-deficient media argue for the existence of a cation efflux, i. e. Na efflux.

We can notice that the tonic tension enhancement in Ca-rich solution (Ca 5) cannot be due to the slight decrease in external sodium concentration (see Material and Methods). Indeed, in a Na-poor medium (NaCl 1/3, LiCl 2/3), the enhancement of tension is transient (15—20 s) and reaches 10 to 17 per cent of the control value; in Ca-rich medium, tonic tension increases by a factor 3.5 to 5 and is maintained at this level as long as the experiment is performed (15 to 20 minutes).

Relation between contractility and excitability

In excitable cells two components of tension may develop (Figs. 3 and 4). In the range of potentials which correspond to the activation of the calcium current and thus in the range of normal excitability of the muscle fibres, the two components are superimposed. For potentials which do not induce the I_{Ca} activation, i. e. for potentials lower than about +30 mV and higher than the reversal potential for Ca ions only the tonic tension develops.

In a proportion of fibres showing well developed electrical activity, the tonic tension is not apparent and the relationship between amplitude of tension and applied potentials is a bell-shaped curve resembling that described in TEA, Na-free medium (Fig. 6B).

In passive fibres the tonic component of tension forms the basis of the mechanical activity. We noticed that in passive fibres, I_{K1} is not always activated. According to Mounier and Vassort (1975b), I_{K1} can be activated when $[Ca]_i$ is enhanced. Following this hypothesis, the variability of I_{K1} activation and its lability can be explained by the fact that, either the $[Ca]_i$ increase, visualized by the tension, or the initial level of $[Ca]_i$ or both can vary from fibre to fibre. Moreover, the $[Ca]_i$ enhancement may have two origins: either the Ca influx linked to the sodium efflux or a very small I_{Ca} through the calcium channel that cannot be detected due to the outward and leak currents.

Mechanical threshold

A mechanical threshold was shown to be dependent on both voltage and duration of membrane depolarization (Fig. 9B) and was always lower than the threshold for I_{Ca} and of the phasic tension. It was related to the treshold for tonic tension. Contrary to the results obtained on the frog heart muscle (Vassort and Rougier 1972), there is always a threshold for this part of mechanical activity. This means that in crab muscle fibre, $[Ca]_i$ at rest is much lower than the $[Ca]_i$ necessary to induce the smallest tension. This was confirmed by the measurements performed in barnacle fibres: the former $[Ca]_i$ was 0.8×10^{-7} mol/l (Ashley and Ridgway 1970), the latter was 5×10^{-7} to 9×10^{-7} mol/l (Hagiwara and Nakajima 1966; Ashley 1967). This means that an enhancement of $[Ca]_i$ by a factor of 6 to 11 is required to

give the mechanical threshold. Thus, a minimum calcium influx due to Na—Ca exchange is necessary to induce a minimum $[Ca]_i$ increase and a tension.

In conclusion, our results show that even if a Ca spike is not required, the presence of external calcium is necessary for the development of a contraction. Two possible Ca influxes were determined, first a Ca influx through the Ca channel, second, a Ca influx probably linked to Na efflux. However, our results do not rule out the possibility of a Ca release from internal stores to induce contraction. We suggest that the apparent contradiction in the results obtained by different authors in other crustacean muscles may result from the relative contribution of each of the Ca influxes to the development of mechanical activity.

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