Is Inward Calcium Current in Crayfish Muscle Membrane Constituted of One or Two Components?

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Abstract. Two inward currents were observed in crayfish muscle membrane during depolarization steps by the method described by Adrian et al. (1970). Under voltage clamp conditions, hyperpolarization steps elicited a large current (leak current I_f), associated with an inward voltage dependent current. This inward current was inhibited by niflumic acid (NA), a drug known to block Cl^-—HCO_3^- exchange (Cousin et Motais 1982; Brûlè et al. 1983b). Dynamic outward currents triggered by depolarizing steps were inhibited to a great extent by TEA, the not inhibited portion disappearing when procaine (2 mmol/l) was added to external solution. In the presence of TEA, procaine and NA, it was thus possible to dissect the regenerative calcium current (I_{Ca}) into two components: a "fast component" (I_{Ca_1}) and a "slow component" (I_{Ca_2}). The reversal potential of I_{Ca} was 65 mV (for $[Ca]_0 = 2.8$ mmol/l), and $[Ca]_i$ could be calculated to be 1.6×10^{-5} mol/l. This value of $[Ca]_i$ is the same as calculated from values reported by Henček and Zachar (1977). I_{Ca_1} was triggered at a threshold membrane potential of -45 mV and I_{Ca_2} at -30 mV. Moreover, the inactivation kinetics for I_{Ca_1} was faster than that for I_{Ca_2}. Our results are in perfect agreement with those obtained by Zahradník and Zachar (1982) who postulated two populations of calcium channels.

Key words: Voltage clamp — Skeletal muscle fibre — Calcium inward currents — Crayfish

Introduction

It is well established that in crustacean fibres, the inward current responsible for the depolarization phase of the response recorded under current clamp conditions is carried by Ca^{2+} ions (Fatt and Ginsborg 1958; Henček et al. 1968, 1972; Haudecoeur and Guilbault 1972; Mounier and Vassort 1973). This current is inhibited by Ca inhibitors as shown by Keynes et al. (1973); Hagiwara et al. (1974) in barnacle fibre ; Reuben et al. (1961) in lobster; Fatt and Ginsborg (1958) Henček and Zachar (1977) in crayfish; Mounier and Vassort (1975) in crab muscle fibre. Under voltage clamp conditions, a decrease in the Ca current during a step

depolarization sufficient to activate the Ca membrane conductance, may be produced: i) by ion accumulation (in barnacle fibre, Keynes et al. 1973); ii) by inactivation of the Ca conductance depending upon voltage (in crab fibre, Mounier and Vassort 1975 and in crayfish fibre, Henček and Zachar 1977). However, results still show discrepancies: the value of the reversal potential for the Ca current in crayfish muscle fibre is 85 mV (Henček and Zachar 1977) while being only 30 mV in crab muscle fibre (Mounier and Vassort 1975). Zahradník and Zachar (1982) have recently described two components of the Ca current in crayfish muscle membrane under conditions in which dynamic currents corresponding to the outward going rectification were abolished; the second component ("slow component") was less sensitive to the duration of the depolarizing prepulse than the first one.

Since the results reported above show some discrepancy, the aim of the present work was to describe the inward calcium current in crayfish muscle membrane under ionic conditions which allow to obtain a pure Ca current, i.e. without contamination by a residual outward current elicited by depolarization steps, using the three-microelectrode voltage clamp technique (Adrian et al. 1970).

The present study deals with the isolation of the Ca current from the total current and with the description of its two components. Our results are in agreement with those obtained, on the same preparation, by Zahradník and Zachar (1982) using the annular sucrose gap method (Henček et al. 1969) and a K^+ free intracellular solution.

Materials and Methods

Dissection of muscle fibres

Experiments were performed on fibres of the extensor muscle isolated from the meropodite of the walking legs of the crayfish. Their diameter varied from 200 to 400 μm. The fibres used in the present experiments had diameters between 250 and 350 μm. They were isolated in physiological solution (VAN HARREVELD solution, for its ionic composition see below) and bathed in this solution before experiments for 15 min for recovery.

Voltage-clamp

The voltage clamp technique used has been described by Adrian et al. (1970). Briefly, the potential difference recorded at distance l from the fibre end is termed E_1. The potential difference across the membrane recorded at $2l$ from the fibre end is E_2. A third microelectrode was used to pass current. It impales the fibre at a distance $2l + l'$ from the fibre end. The membrane current density corresponding to the membrane potential (E_1) is given by the following equation: $I_m\ (A\ .\ cm^{-2}) = [d(E_2 - E_1)]/6R_i l^2$, where R_i is the internal resistivity taken as 125 Ω . cm at 20 °C (Fatt and Ginsborg 1958) and d is the diameter of the fibre.

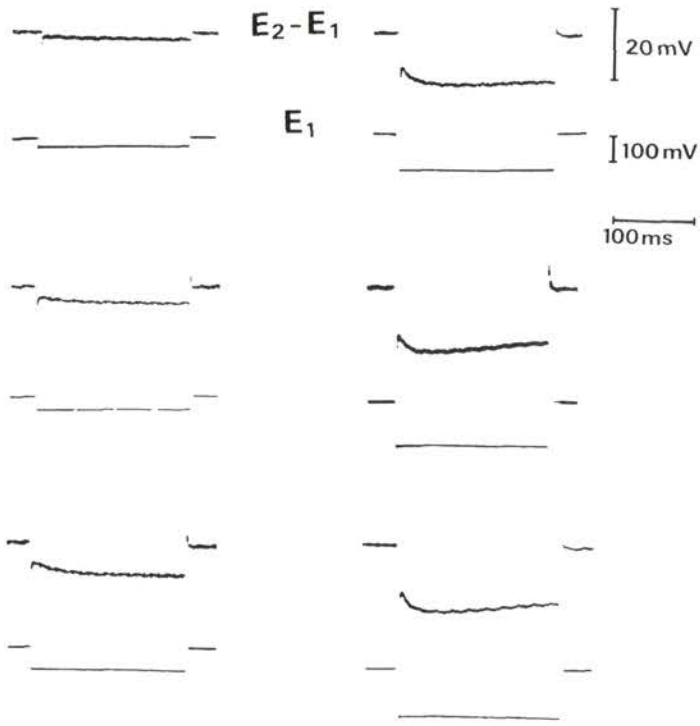

Fig. 1. Each pannel shows tracings of membrane current in terms of potential difference (E_2-E_1) (upper traces) and membrane potential E_1 (lower traces) under voltage clamp and normal ionic conditions. (E_2-E_1) is proportional to membrane current density. Fibre diameter $(d)=0.025$ cm; $l=0.025$ cm; $l'=0.025$ cm; Resting potential (RP) $=-60$ mV; Holding potential (HP) $=-70$ mV. 20 mV for $(E_2-E_1)\equiv 1.06\times 10^{-3}$ A . cm^{-2}.

Results and Discussion

I — *Inhibition of the inward and outward going rectifications*

Before the isolation of the Ca current components from the total membrane current elicited by depolarization steps, it was necessary, as mentioned above, to block the dynamic inward and outward currents produced by hyperpolarizing and depolarizing pulses respectively. Indeed, as indicated by Hagiwara et al. (1969), Henček et al. (1978), and Zahradník and Zachar (1982), the Ca current may be contaminated by some dynamic outward currents. Moreover, the presence of the inward going rectification does not allow to obtain extrapolated leak current for each value of depolarizing pulse from the current-voltage relationship established from current measured for different hyperpolarizing pulses (the total current varies with time).

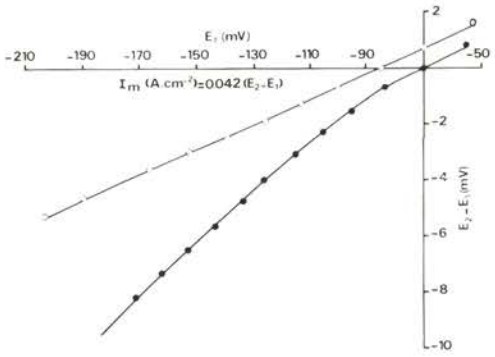

Fig. 2. Current-voltage relationships measured at the end of 200 ms voltage steps on a fibre bathed in VH solution (●) and in VH solution containing niflumic acid [10^{-6} mol/l (○)]. $d = 0.031$ cm; $l = 0.031$ cm; $l' = 0.031$ cm in VH solution: RP = HP = −70 mV; in VH–NA solution: RP = HP = −86 mV.

Inhibition of the inward going rectification

An example of tracings of imposed hyperpolarizing pulses (E_1) and the corresponding potential differences (E_2—E_1), proportional to the membrane current, for a crayfish muscle fibre bathed in normal ionic solution (Van Harreveld Solution; Solution I) is illustrated in Fig. 1. Membrane current corresponding to the leak current is associated with an inward current elicited by activation of the inward going rectification.

This inward going rectification did not disappear following the addition to the normal solution of TEA or by substituting K^+ ions by Cs^+ ions. By contrast, as shown in Fig. 2, inward going rectification disappeared when NA at a 10^{-6} mol/l concentration was added to the extracellular solution. In the presence of NA, the slope of the current-potential curve did no more increase with the amplitude of the hyperpolarizing pulse. The current corresponding to this inward going rectification is carried by Cl^- ions, since it has been demonstrated in the red blood cell membrane that this amphiphilic agent inhibits $Cl^- - CO_3H^-$ exchange (Cousin and Motais 1979, 1982). Detailed results concerning inhibition of the inward going rectification by NA in crayfish muscle fibre were reported previously (Brûlé et al. 1983b).

Thus in the presence of NA, the leak current value of depolarizing pulses can be extrapolated from the linear current-voltage relationship established using hyperpolarizing pulses.

Inhibition of the outward going rectification

Henček and Zachar (1977) and Henček et al. (1978) have described three outward

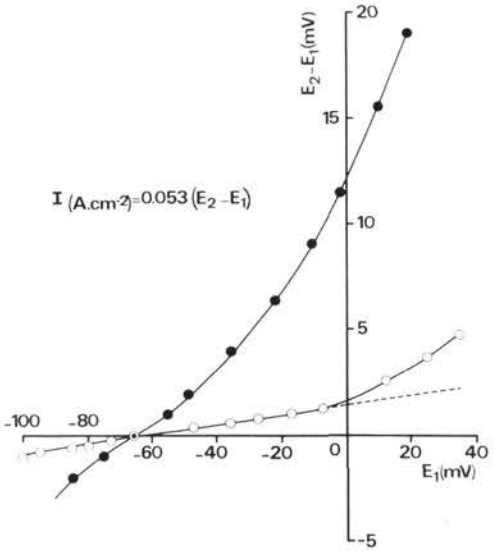

Fig. 3. Current-voltage relationships measured at the end of 500 ms voltage steps in VH solution containing Co^{2+} ions (10 mmol/l) (● solution III) and in VH—Co^{2+} solution in the presence of TEA (60 mmol/l) and NA (10^{-3} mmol/l) (○ solution IV). $d = 0.024$ cm; $l = 0.025$ cm; $l' = 0.025$ cm. In sol. III and IV: RP = HP = −66 mV.

currents elicited in crayfish muscle membrane by imposed steps of depolarization. These currents disappeared in the presence of TEA ions (100 mmol/l) known to block K currents. However, Zahradník and Zachar (1982) perfused crayfish fibres with K free intra- and extracellular solutions in order to eliminate completely the outward current. Under these particular ionic conditions, these authors could describe two components of the inward Ca current. Moreover, Hagiwara et al. (1969) showed in barnacle muscle membrane that the reversal potential of the regenerative Ca inward current was shifted in the depolarization direction when a component of the outward current insensitive to TEA was eliminated by procaine.

In crayfish muscle membrane, in the presence of cobalt ions which abolish Ca current, TEA^+ ions produce a large, but incomplete, decrease of the outward current as shown in Figure 3.

II — *Analysis of the calcium current*

For technical reasons (e.g. microelectrode breaking), contractions need to be avoided. This is obtain by adding to the bath Mn^{2+} ions (2 mmol/l) or procaine (2 mmol/l). Moreover, Mn^{2+} ions or a low Ca in solution containing procaine

Table 1. The composition of external solutions used in the present experiments. The values are given in mmol/l.

№	Solutions	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Other substances
I	Normal Solution VAN HARREVELD (VH)	212.1	248.8	5.25	14	2.8	2.1	—
II	VH niflumic acid (VH–NA)	212.1	248.8	5.25	14	2.8	2.1	niflumic acid = 10^{-3}
III	VH–Co^{2+}	192.1	248.8	5.25	14	2.8	2.1	Co^{2+} = 10
IV	VH–Co–TEA–NA	132.1	248.8	5.25	14	2.8	2.1	Co^{2+} = 10 TEA^+ = 60 NA = 10^{-3}
V	VH–TEA–NA–Mn^{2+}	159.3	248.8	5.25	14	2.8	2.1	TEA^+ = 60 Mn^{2+} = 2 NA = 10^{-3}
VI	VH–TEA–1/5 $[Ca]_o$ –NA-procaine	174.4	248.8	5.25	2.8	2.8	2.1	TEA^{2+} = 60 NA = 10^{-3} Procaine = 2

(2 mmol/l) allowed to obtain a better voltage control by decreasing the Ca current amplitude.

1. *Mn^{2+} ions*

For the composition of the solution used see Table 1 (Solution V). The presence of TEA^+ ions, NA and Mn^{2+} ions (VH — Mn — TEA — NA) resulted in the inhibition of the inward going rectification (Fig. 2) and a large part of the outward current (Fig. 3), respectively, triggered by hyperpolarizing and depolarizing steps. The residual part of this outward current was activated at a threshold membrane potential of -10 mV. This TEA insensitive outward current significantly contamined the Ca current corresponding to values of membrane potential up to 0 mV; this outward current is carried by K^+ and/or Cl^- ions (Hagiwara et al. 1969; Brûlé et al. 1983a). In fibres bathed in solution V, membrane current typically varied as a function of the amplitude of a step, as illustrated in Fig. 4. The early inward current was activated by potentials (E_1) positive to -45 mV. At membrane potential of 0 mV, the current was net inward and maintained constant throughout the depolarization step (180 ms). With a large depolarization ($E_1 = 28$ mV), the total current nearly kept its maximal amplitude during the depolarization step, but it became an outward current. Finally, with an even more large depolarization step

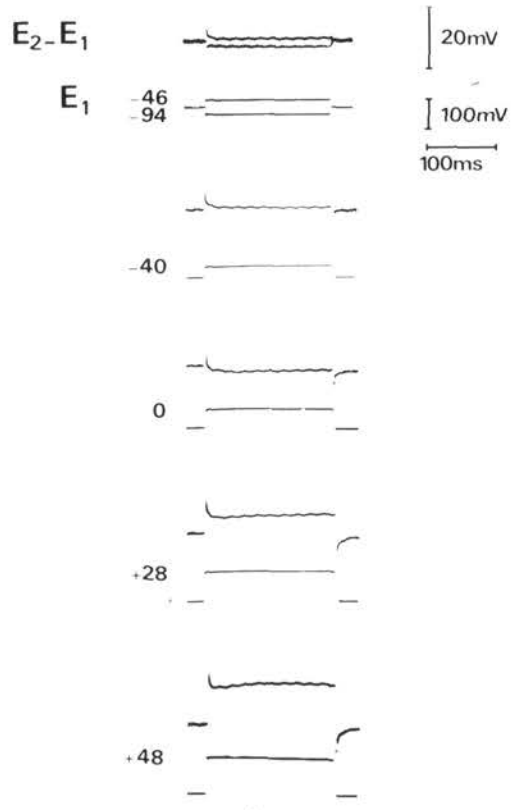

Fig. 4. Membrane current expressed in terms of $(E_2 - E_1)$ (upper traces) and corresponding membrane potential E_1 (lower traces) under voltage clamp conditions, for a fibre bathed in VH solution containing Mn^{2+} ions (2 mmol/l) TEA ions (60 mmol/l) and NA (10^{-3} mmol/l) (solution V). $d = 0.025$ cm; $l = 0.025$ cm; $l' = 0.025$ cm. RP $= -60$ mV. HP $= -70$ mV. 20 mV for $(E_2 - E_1) \equiv 1.06 \times 10^{-3}$ A . cm^{-2}. Imposed membrane potentials (E_1) are indicated on the left of each pannel.

($E_1 = 48$ mV), the total outward current slowly increased with time. This time dependent increase of the outward current can be explained i) by a decrease of the Ca current with time ii) by the presence of the slowly activated residual outward current insensitive to TEA (Fig. 3).

The measure of the maximal inward or minimal outward current gives a total current-voltage relationship (Fig. 5) with the more negative value of current corresponding to membrane potential 0 and a reversal potential of 10 mV. The inhibition of the inward going rectification by NA resulted in a linear $I - V$ relationship for the values of E_1 more negative than -50 mV. From this linear relation, the different values of the leak current corresponding to depolarizing

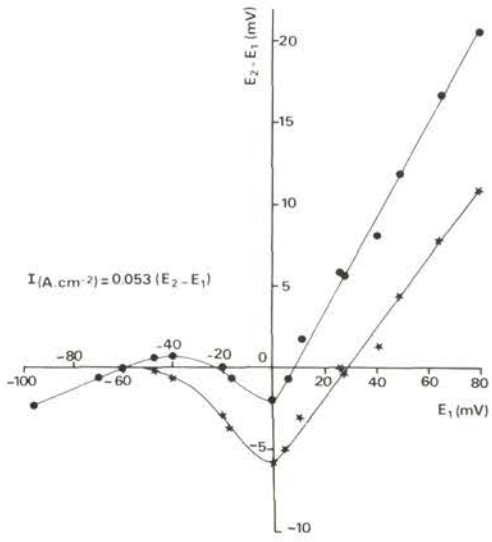

Fig. 5. Current-voltage relationships constructed from tracings shown in Figure 4: (●) Peak inward total current; (I) Early inward current obtained by substracting the extrapolated leak current from the total current.

pulses could be extrapolated. The current-voltage relationship plotted, with a correction for the leak current, showed that inward current initiated from −45 mV reached its maximal value at 0 mV and reversed at 30 mV. Above 0 mV, this current, supposed to be a pure calcium current, varied linearly with voltage.

The low value of the reversal potential of the calcium current in our experiment (30 mV), being quite different from 85 mV established by Henček and Zachar (1977), and the linear I_{Ca}-voltage relation above 0 mV, contrasting with the rectification of I_{Ca} observed by several others (see Hagiwara et Byerly 1981) can be explained by an overlapping outward current.

2. *Procaine*

For the composition of the procaine containing solution, see Table 1 (solution VI). Under these conditions, inward and outward going rectifications were completely abolished and a pure Ca current large enough to be analysed was recorded despite a reduced concentration of Ca^{2+} ions ($1/5\ [Ca]_0$). Moreover, this Ca current elicited by depolarizing pulses was unable to trigger muscle contraction.

Figure 6 shows an example of tracings recorded in muscle fibre bathed in solution VI. For each level of imposed depolarization, membrane current is equal to the sum of I_{Ca} and I_f. For an imposed membrane potential of −35 mV, membrane current was somewhat inward at the beginning of the depolarizing

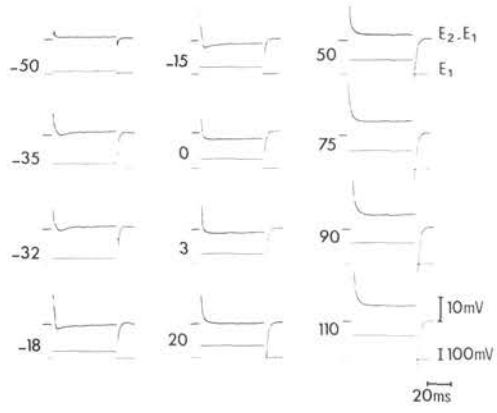

Fig. 6. Membrane current in terms of $(E_2 - E_1)$ (upper traces) and corresponding membrane potential E_1 (lower traces) under voltage clamp conditions, for a fibre bathed in poor Ca (1/5 $[Ca]_o$) VH solution containing TEA ions (60 mmol/l), NA (10^{-3} mmol/l) and procaine (2 mmol/l) (solution VI). $d = 0.031$ cm; $l = 0.031$ cm; $l' = 0.031$ cm; RP = −60 mV; HP = −70 mV. Imposed membrane potentials (E_1) are indicated on the left of each pannel.

pulse, then it decreased as a function of time and became outward at the end of the pulse. This diminution can be due to an inactivation process depending upon both voltage and time. With a depolarizing pulse of 55 mV ($E_1 = -15$ mV), the membrane current was inward from the beginning until the end of the step. However, it remained time dependent. By contrast, for E_1 from 0 mV to 40 mV, membrane current reached rapidly a maximal amplitude and remained constant, thereafter; thus, no inactivation process was apparent. This inward current reversed at membrane potential of about 20 mV. This discrepancy between large and low depolarizing pulse effects led us to propose a mechanism for the inactivation process other than a simple voltage and time dependence.

Recently, another process of inactivation has been described by several authors. According to their suggestions, inactivation would be dependent upon the quantity of Ca^{2+} ions which enter the fibre via the Ca channels (Brehm and Eckert 1978; Ashcroft and Stanfield 1980). This explanation is not sufficient to explain our records illustrated in Figure 7, where the maximal amplitude of I_{Ca} (for $E_1 = 0$ mV), did not decrease as a function of time. Our results could be better explained by the existence of two components in the Ca current, as proposed by Zahradník and Zachar (1982). The time course of I_{Ca} shown in Figure 7 for several depolarization amplitudes from the holding potential of −70 mV supports the hypothesis concerning the existence of two populations of Ca channels. For $E_1 = -35$ mV, the current showed inactivation which was completed within 20 ms. If inactivation were voltage and time dependent it would be difficult to explain why

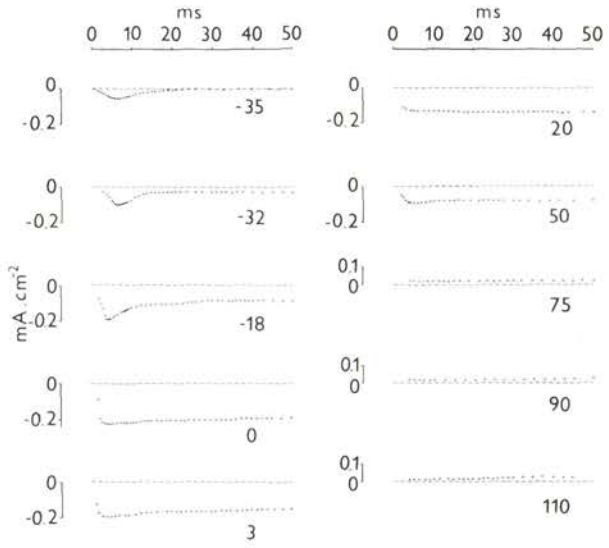

Fig. 7. Dissection of inward Ca current from the total membrane current. Each tracing gives time course of I_{Ca} corresponding to a value of the membrane potential, E_1 indicated below the traces. These I_{Ca} are derived from total currents illustrated in Figure 6 by subtracting capacitive and leak currents.

a one component Ca current remains constant throughout the application of pulses at membrane potentials exceeding or equal to 0 mV. Ca current remaining constant and reaching its maximal value at a zero membrane potential, where the Ca dependent inactivation process should be most marked, reinforces the above hypothesis. Furthermore, Ca current-voltage curves (Fig. 8) drawn from tracings shown in Fig. 7 indicate that neither inactivation depending on voltage nor a calcium entry can explain why these curves are different for low, but not for large, depolarizing pulses. It should be noted that, when the membrane potential is near the I_{Ca} reversal potential, the I_{Ca}-voltage relationship rectifies (Fig. 7, 8) as previously reported by Ashcroft and Stanfield (1980) for insect muscle fibre.

3. *Procaine and a conditioning prepulse*

At a holding potential of -45 mV, no inward Ca current could be triggered in crayfish muscle membrane, even with large depolarization; however, with a sequential holding potential of -70 mV inward Ca current was activated. This seems to suggest that the two I_{Ca} components would be inactivated by the same process (voltage and time dependent inactivation). Indeed, the tracings in Figure 9 suggest that after a large conditioning depolarizing pulse an inward current appears during the test pulse, it is voltage and time dependent; this current corresponds to

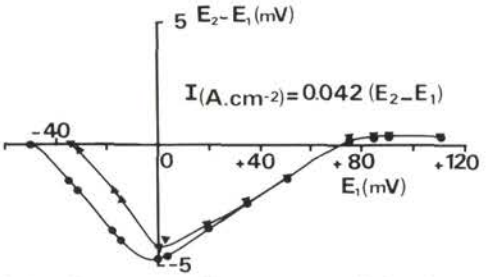

Fig. 8. Current-voltage relationships of the peak inward current (●) and current measured at the end of 50 ms voltage steps (▲). Curves are constructed with a correction for the leak current from tracings shown in Figure 6.

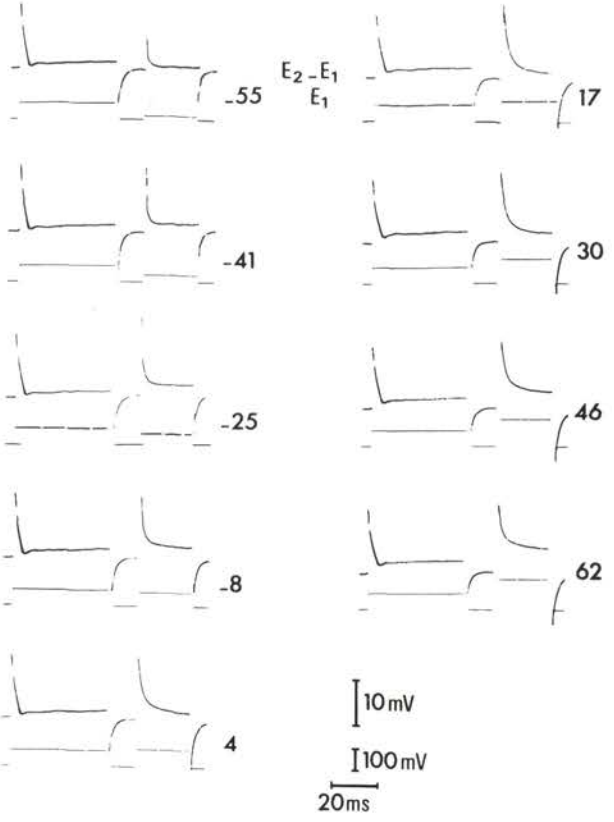

Fig. 9. Each pannel corresponds to tracings of membrane current expressed in terms of $(E_2 - E_1)$ (upper traces) and imposed membrane potential E_1 (lower traces). Membrane current was recorded for a large depolarizing prepulse (50 ms) followed by a test pulse of different values indicated on the right of each pannel. Recording were made in crayfish muscle fibre bathed in solution VI (VH − TEA − NA − procaine). $d = 0.044$ cm; $l = 0.044$ cm; $l' = 0.044$ cm; RP = HP = −70 mV. 10 mV for $(E_2 - E_1) \equiv 0.3 \times 10^{-3}$ A . cm^{-2}.

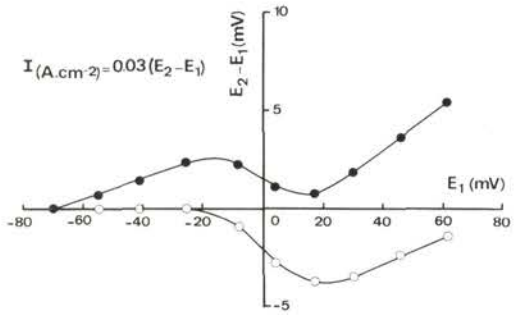

Fig. 10. Current-voltage relationships constructed from tracings shown in Figure 9 at the end of test pulses without correction for leak current I_f (●) and after substraction of I_f (○).

the second I_{Ca} with slow inactivation kinetics. This result is summarized in Figure 10, where the curve corresponding to I_{Ca} measured at the end of the test pulses as a function of E_1 shows that the slow inward current is activated at a threshold membrane potential of -25 mV and reaches its maximal value at $E_1 \simeq +20$ mV.

Conclusion

The results presented in this paper (Fig. 6, 7, 8, 9 and 10) lead us to conclude, in agreement with the results of Zahradník and Zachar (1982), that two Ca currents exist in crayfish muscle membrane. These two components can be separated according to their different inactivation kinetics and their threshold activation potential.

Inward calcium currents recorded under voltage conditions in intact crayfish muscle fibre are difficult to analyse, due to the existence of outward current difficult to block completely from the external side of the membrane. Moreover, the value can not be extrapolated without the inhibition of the inward going rectification of the leak current. The current-voltage relationship curve can be established after the blockade of the inward going rectification for various hyperpolarizing pulses. These problems were avoided using niflumic acid to inhibit inward current activated by hyperpolarizing voltage steps (Brûlé et al. 1983b), and TEA ions plus procaine to eliminate voltage and time-dependent outward currents. For all the amplitudes of depolarization, membrane current recorded under voltage clamp conditions is the sum of inward Ca current and leak current. With the microelectrode technique described by Adrian et al. (1970) which is also suitable to analyse membrane current in crustacean muscle fibre (Bertrand et al. 1979; Brûlé et al. 1983a), it has been possible to dissect the Ca current in crayfish muscle membrane.

The results reported in this paper suggest the existence of two components of the regenerative inward current carried by Ca^{2+} ions. In our approach the problem concerning the muscle activation was solved by using a Ca poor solution (1/5 $[Ca]_0$). A diminution of the extracellular concentration of calcium ions also allowed a better control of the voltage. The two components of the Ca inward current could be distinguished based on their different activation and inactivation kinetics since depolarization steps elicited an early and a late inward current. The early current (I_{Ca_1}) is activated at a threshold membrane potential of about -45 mV and the late inward current (I_{Ca_2}) at about -30 mV. These currents disappeared in VH solution containing Co^{2+} ions (Fig. 3).

I_{Ca_1} and I_{Ca_2} could be separated by applying a large conditioning prepulse of a 50 ms duration. Under this condition I_{Ca_1} was rapidly inactivated whereas I_{Ca_2} could still be observed during depolarizing test pulses. Inactivation of I_{Ca_2} seemed to be voltage and time dependent, similarly as that of I_{Ca_1} because I_{Ca_1} and I_{Ca_2} could not be activated when crayfish muscle membrane was depolarized by 20 mV from a resting potential of -70 mV; however, such a depolarization was unable to trigger a regenerative inward current. Under these conditions, inactivation of calcium conductance would not be mediated by Ca^{2+} ions which enter the fibre through the Ca channels (Tillotson 1979).

Moreover, an analysis of activation of calcium channels corresponding to I_{Ca_1} implies the displacement of 6 particles in the membrane to open the corresponding channel, whereas 2 particles only are necessary to open the channel corresponding to I_{Ca_2} (unpublished results, paper in preparation).

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References

Adrian R. H. Chandler, W. K., Hodgkin A. L. (1970): Voltage clamp experiments in striated muscle fibers. J. Physiol. (London) **175**, 134—159

Ashcroft F. M., Stanfield P. R. (1980): Inactivation of calcium currents in skeletal muscle fibres of an insect depends on calcium entry. J. Physiol. (London) **308**, 36 P

Bertrand F., Brûlé G., Guilbault P., Haudecoeur G. (1979): Expériences de potentiel imposé à l'aide de microélectrodes sur la fibre musculaire squelettique de crabe. Etude du courant membranaire global. J. Physiol. (Paris) **263**, 251—252

Brehm P., Eckert R. (1978): Calcium entry leads to inactivation of calcium channel in *Paramecium*. Science N.Y. **202**, 1203—1206

Brûlé G., Haudecoeur G., Guilbault P. (1983a): Ionic nature of the outward going rectification in crab skeletal muscle fibres. Gen. Physiol. Biophys. **2**, 137—151

Brûlé G., Haudecoeur G., Jdaïâa H., Guilbault P. (1983b): Inhibition par une substance amphiphile: l'acide niflumique de la rectification dans le sens entrant de la fibre musculaire de crustacé. Arch. Int. Physiol. Biochim. **91**, 269—277

Cousin J. L., Motais R. (1979): Modification de la perméabilité des membranes biologiques par des composés amphiphiles d'intérêt pharmacologique. Colloques nationaux du CNRS № 938, Physicochimie des composés amphiphiles, p. 243

Cousin J. L., Motais R. (1982): Inhibition of anion transport in the red blood cell by anionic amphiphilic compounds. II; chemical properties of the flufenamate-binding site on the band 3 protein. Biochim. Biophys. Acta, **687**, 156—164

Fatt P., Ginsborg B. L. (1958): The ionic requirements for the production of action potentials in crustacean muscle fibres. J. Physiol. (London) **142**, 516—543

Hagiwara S., Byerly L. (1981): Calcium channel. Annu. Rev. Neurosci. **4**, 69—125

Hagiwara S., Hayashi H., Takahashi K. (1969): Calcium and potassium currents of the membrane of a barnacle muscle fiber in relation to the calcium spike J. Physiol. (London) **205**, 115—129

Hagiwara S., Fukuda J., Eaton D. C. (1974): Membrane currents carried by Ca, Sr and Ba in barnacle muscle fiber during voltage clamp. J. Gen. Physiol. **63**, 564—578

Haudecoeur G., Guilbault P. (1972): Contribution à l'étude de l'activité électrique de la fibre musculaire striée de crabe (Carcinus maenas). I. Effets sur le potentiel d'action de solutions de contenu ionique modifié. J. Physiol. (Paris) **64**, 367—386

Henček M., Zachar J. (1977): Calcium currents and conductances in the muscle membrane of the crayfish. J. Physiol. (London) **268**, 51—71

Henček M., Zachar J., Zacharová D. (1968): Inward currents in the muscle membrane with graded electrogenesis. Physiol. Bohemoslov. **17**, 464—465

Henček M., Nonner W., Stämpfli R. (1969): Voltage clamp of a small muscle membrane area by means of a circular sucrose gap arrangement. Pflügers Arch. **313**, 71—79

Henček M., Zachar J., Zacharová D. (1972): Voltage clamp experiments on single fibers with graded electrogenesis. Abstr. IVth int. Biophysical Congress Moscow, EXII–XIII a 29

Henček M., Zachar J., Zacharová D. (1978): Membrane currents in a calcium type muscle membrane under voltage clamp. Physiol. Bohemoslov. **27**, 457—466

Keynes R. D., Rojas E., Taylor R. E., Vergara J. (1973): Calcium and potassium systems of a giant barnacle fibre under membrane potential control. J. Physiol. (London) **229**, 409—455

Mounier Y., Vassort G. (1973): Analyse en potentiel imposé des courants membranaires de la fibre musculaire de crabe C. R. Acad. Sci. Paris, **276**, 173—176

Mounier Y., Vassort G. (1975): Initial and delayed membrane currents in crab muscle fibre under voltage-clamp conditions. J. Physiol. (London) **251**, 589—608

Reuben J. P., Werman R., Grundfest H. (1961): The ionic mechanisms of hyperpolarizing responses in lobster muscle fibers. J. Gen. Physiol. **45**, 243—265

Tillotson D. (1979): Inactivation of Ca conductance dependent on entry of Ca ions in molluscan neurons. Proc. Nat. Acad. Sci. USA **76**, 1497—1500

Zahradník I., Zachar J. (1982): Calcium currents in the muscle membrane of the crayfish in K^+-free internal environment. Gen. Physiol. Biophys. **1**, 457—461

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