Short communication

Three Components of Calcium Currents in Crayfish Skeletal Muscle Fibres

M. HENČEK and D. ZACHAROVÁ

Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Czechoslovakia

The coexistence of multiple types of calcium currents within the same cell has been demonstrated in voltage clamp experiments in several cells (for review see Hagiwara et Byerly 1981). Single channel recordings confirmed the existence of distinct electrical and pharmacological properties of Ca channels and, based on these differences, three types of Ca channels were characterized (classified) as separate entities (for review see Bean 1989). So far, two Ca currents have been described to exist in muscle fibres, namely in those spiking on calcium principle. On internally perfused muscle fibre segments of the crayfish Zahradník and Zachar (1982) described two components of Ca currents with different kinetics of inactivation. Detailed electrophysiological analyses of the two Ca current components (Zahradník 1985, Jdaiaa and Guilbault 1986, Zahradník and Zachar 1987) support the hypothesis concerning the existence of two different populations of Ca channels in these muscle fibres.

Another support for the existence of multiplicity of calcium channels in these membranes came from studies on isolated and purified calcium channel membrane proteins. Recently a DHP binding protein was isolated and characterized from the T-tubules of the crayfish (Križanová et al. 1990), and the corresponding conductance of the DHP sensitive Ca channels incorporated into planar bilayers was measured (16 pS) by Hurňák et al. (1990). Two Ca channels were expressed in *Xenopus* oocytes injected with crab skeletal muscle fibre mRNA (Fournier et al. 1990). Quite recently the activity of two types of Ca channels (14 and 38 pS) in the plasma membrane of the crayfish tonic flexor muscle was reported (Bishop et al. 1991).

To further differentiate the properties of fast and slow Ca channels in the muscle membrane of the crayfish and to investigate whether these two channels resemble those reported in other tissues, we examined the effects of low Ca^{2+} solution, the substitution of Sr^{2+} and Ba^{2+} ions for Ca^{2+} as charge carriers, and the effects of inorganic channel blockers Ni^{2+} and Cd^{2+}.

Three Ca dependent components were observed to exist in most muscle segments at negative depolarizations, differing in inactivation characteristics and susceptibility to the block by inorganic cations.

The experiments were performed on internally perfused muscle segments of the m. extensor carpopoditi of the crayfish *Astacus fluviatilis*. Vaseline gap voltage clamp method (Hille and Campbell 1976) was used to record Ca currents. The composition of the internal solution was (in mmol/l): 240 Cs- glutamate; 1.0 $MgCl_2$; 0.01 Ca-glutamate; 10 EGTA; 5 ATP; 0.2 cAMP; 10 HEPES; pH 7.3. The external (control) solution contained: 208 TMA-glutamate; 13.5 Ca-glutamate; pH 7.3.

Ionic currents were recorded after analog compensation for the leak and capacitance components and filtration with a 10 kHz low pass filter. The records were stored on magnetic medium using PMD 85 microcomputer (TESLA, ČSFR). Isolation of the individual current components (differing in their time courses of activation and inactivation) from total calcium current, based on the Hodgkin - Huxley model for conductances (Hodgkin and Huxley 1952), was performed using a laboratory software (Pavelková et al. 1991). Exponent 6 instead of 3 in the H-H equation for m variable was used for calculation of the calcium conductance (Henček and Zachar 1977). In order to evaluate amplitude and time parameters of a particular component the currents were transformed to conductances in mS/cm^2 assuming the equilibrium potential of + 50 mV. The time course of the calcium

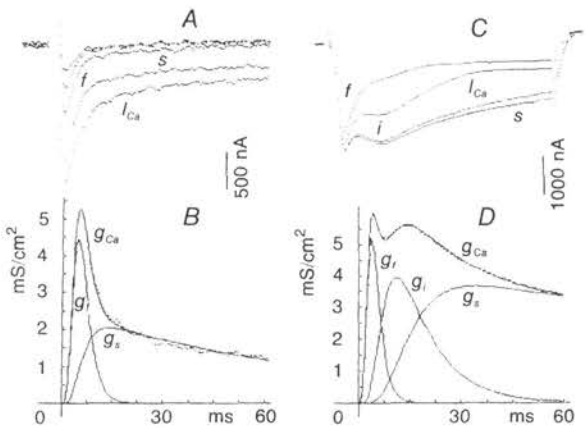

Figure 1. Total calcium currents, I_{Ca} (A, C) and conductances, g_{Ca} (B, D) from two crayfish muscle fibre segments. Inward calcium currents are oriented downwards (f, i, s are suggested current components). The dotted curves stand for original records; the full lines are computed drawings. The individual calcium conductances, g_f (fast), g_i (intermediate), g_s (slow) were dissected from the total calcium conductance g_{Ca} using H-H equations as described in text. The ionic currents were evoked by a series of depolarizing pulses starting from the holding potential of −80 mV.

conductance, g_{Ca} was fitted by summing three equations with nine parameters:

$$g_{Ca} = g_f\,(1 - \exp(-t/\tau_{mf}))^6 \exp(-t/\tau_{hf}) + \\ + g_i\,(1 - \exp(-t/\tau_{mi}))^6 \exp(-t/\tau_{hi}) + \\ + g_s\,(1 - exp(-t/\tau_{ms}))^6 \exp(-t/\tau_{hs}),$$

where g_f, g_i, g_s are conductances of the individual calcium current components; and τ_{mx}, τ_{hx} are the corresponding time constants of activation and inactivation, where x stands for f, i and s respectively. The area under the time course of a particular conductance was evaluated by numerical integration.

Fig. 1 shows two types of multicomponent total calcium currents I_{Ca}, which were recorded from two different muscle fibre segments. Fig. 1*A* illustrates currents to a series of depolarizing pulses (range 40–70 mV) from the holding potential, V_H −80 mV. At low depolarizations (40, 44 mV) first appeared the fast calcium current component (f), with a typically fast inactivation. The second non-inactivating component (s) became apparent at higher depolarization steps (50, 60, 70 mV). The conductances can be derived from these current components by means of H-H equations as shown in (B). The other segment (C) shows three components already at the depolarization step to −50 mV: a fast inactivating (f), a partially inactivating (i) (i for intermediate), and a slowly inactivating component (s). The corresponding conductances derived from the total calcium conductance are shown in (D).

The time course of dissected conductances may be compared with the time course of individual calcium conductances which happen to manifest themselves in few instances separately (Fig. 2). Fig. 2*A* shows the time course of the fast calcium current to a 50mV depolarization step (applied for 3 ms). Another segment (B) with a poorly developed fast component shows the intermediary current in isolation; its inactivation phase is, however, partially contaminated by the slow calcium current component. On the third segment (C) a slow calcium current is shown to a 600 ms pulse. The calcium conductances determined from these separate records (B, D, F) are similar to those dissected from the total calcium conductances (Fig. 1). Time constants of activation (τ_m) and inactivation (τ_h) of the individual calcium conductances determined at membrane depolarization of −30 mV (V_H = −80 mV) are significantly different from each other. Their mean values were as follows: g_f: $\tau_m = 1.59 \pm 0.13$ ms, $\tau_h = 2.03 \pm 0.13$ ms; g_i: $\tau_m = 4.15 \pm 0.54$ ms, $\tau_h = 9.5 \pm 1.09$ ms; g_s: $\tau_m = 7.3 \pm 0.55$ ms, $\tau_h = 460 \pm 41.1$ ms.

After withdrawal of calcium ions from the extracellular medium or a decrease of $[Ca]_0$ to 100 μmol/l, the fast calcium conductance is substantially decreased (Fig. 3*B*; *f*). The intermediary calcium conductance (Fig. 3*B*; *i*) is much less affected and the slow calcium conductance (s) is facilitated. Mean values after 30 s in a low Ca^{2+} solution are as follows: $g_f = 73.3 \pm 2.6\%$, $g_i = 92.6 \pm 2.5\%$,

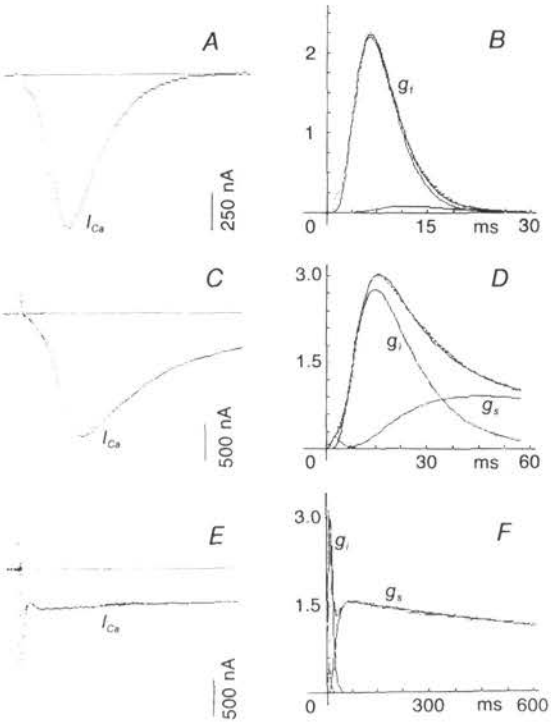

Figure 2. Calcium currents (left panel) and the corresponding conductances (right panel). g_f - fast; g_i - intermediate; g_s - slow conductance. Note the relatively small contamination of the fast calcium conductance (B) with g_i and g_s conductances; and of the intermediate conductance (D) with g_f and g_s conductances. For further explanation see text.

$g_s = 108.7 \pm 4.6\%$; the differences between g_f and g_i or g_f and g_s respectively are statistically significant ($P < 0.001$ and 0.02 respectively). After exposure to low Ca solutions (for 1 to 1.5 min) all Ca conductances decreased ($g_f = 51.2 \pm 2.4\%$, $g_i = 58.8 \pm 3.7\%$, $g_s = 63.3 \pm 7.5\%$; $n = 6$), or practically closed after a longer exposure. After reintroduction of Ca containing solutions (Fig. 3. E, F) the fast calcium component was the first to recover; $g_f > g_i$ ($P = 0.02$); $g_f > g_s$ (0.05).

It is tempting to explain these differences by different distribution of individual calcium channels in the crayfish muscle membrane system. The fast channels are expected to be localized in the surface membrane, the intermediate and the slow ones in the transverse tubular system. It is to be noted that recently a slow calcium channel was identified in the tubular membrane fraction of the crayfish both by biochemical and biophysical methods (Križanová a spol. 1990).

To compare the properties of the proposed calcium channels with the properties of Ca channels denoted as T, N, and L (Nowycky et al. 1985) and identified in different cell membranes (Bean 1989), we examined the effects of divalent cations (Sr^{2+}, Ba^{2+}, Ni^{2+}, Cd^{2+}) on the individual calcium current components.

These cations act differently—though not specifically— on individual calcium channels. Typical changes to agonist and antagonist divalent cations are shown in Fig. 4. Calcium conductances g_i and g_s increase in Sr^{2+} solutions (13.5 mmol/l) substantially more (456.9 ± 145.7) than g_f calcium conductance (143.9 ± 26.9%; $n = 6$, $P = 0.05$). The effect of Ba^{2+} ions was very similar (not shown).

Ni^{2+} ions (4 mmol/l) when applied for the same time interval decrease preferentially g_f and g_i calcium conductances (to 69.3 ± 5.6% and 71.1 ± 5.7% of the control value respectively); g_s was decreased to 91.2 ± 8.6% only. The differences are significant ($P < 0.01$; $n = 17$). Cd^{2+} ions are more potent blockers than Ni^{2+} ions; their blocking effect is already apparent at one order lower concentrations. The effect of 200 μmol/l Cd^{2+} ions on individual calcium conductances is shown in Fig. 4 (third row). The decrease of g_i and g_s is more pronounced than that of g_f. The mean values (after 2 to 5 min in the test solution; 7 segments) are as follows: $g_f = 74.6 \pm 5.5\%$, $g_i = 60.9 \pm 6.6\%$ ($P < 0.001$), $g_s = 66.1 \pm 5.6\%$ ($P < 0.01$).

It follows from these experiments that the three-component calcium current, which is very often observed in crayfish muscle fibre segments, can be explained by assuming three calcium conductances as evidenced by mathematical dissection of the fast, intermediate and slow calcium conductances. This assumption is sup-

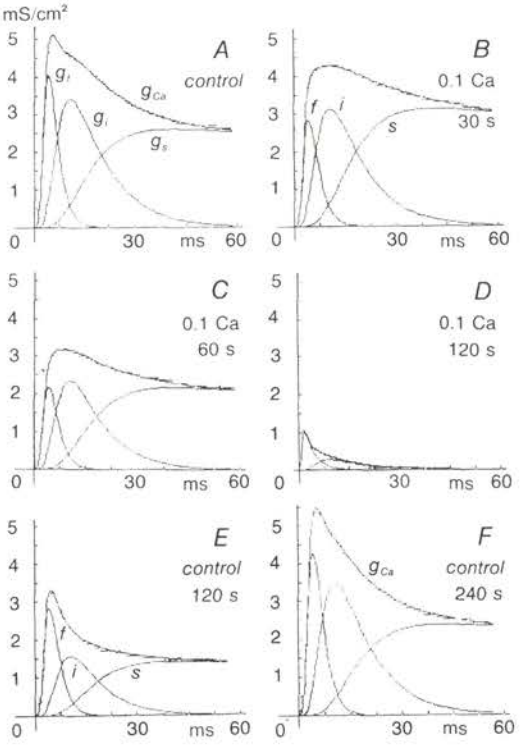

Figure 3. The effect of low external calcium concentration (100 μmol;l) on calcium conductance components. *A*: control (Ca^{2+} = 13.5 mmol;l); *B*: 30 s; *C*: 1 min; *D*: 2 min after introduction of low Ca^{2+} solution; *E*: 2 min; *F*: 4 min after reintroduction of control Ca saline.

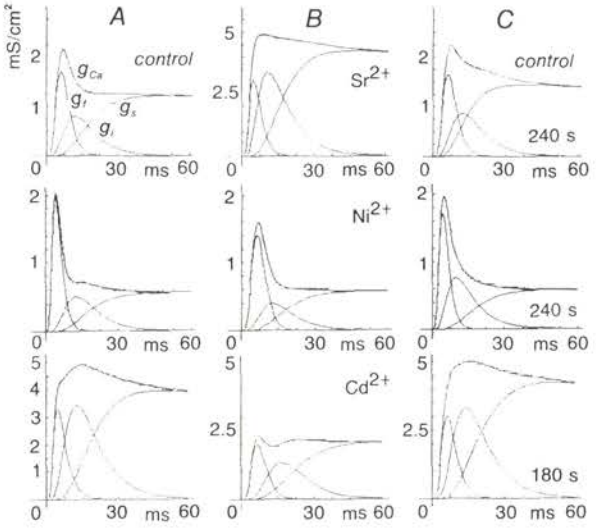

Figure 4. The effect of divalent cations on the calcium current components. Upper row: Sr^{2+} (13.5 mmol;l); middle row: Ni^{2+} (4 mmol;l); bottom row: Cd^{2+} (0.2 mmol;l). *A*: controls (Ca^{2+} = 13.5 mmol;l); *B*: the effects of the divalent cations tested upon their application for 3 min (B1) and 4 min (B2, B3); *C*: recovery after reintroduction of control Ca^{2+} saline.

ported by the existence of the isolated fast calcium current at low depolarizations and especially of the intermediary Ca current in segments lacking the fast Ca current component. The existence of three types of Ca channels in this muscle preparation is further supported by the effects of Ca channel agonists and antagonists, which were used to compare the three types of muscle calcium conductances with three types of calcium channels (T, L, N) present in other structures (Fox et al. 1987).

Significant differences were, however, always found between fast and intermediary and/or slow channels. The differences between intermediary and slow channels (except the effect of Ni^{2+} ions) were not statistically significant. This compares fairly well with the differences between N and L channels, which react in similar way to the action of Sr^{2+}, Ba^{2+}, Ni^{2+} and Cd^{2+} ions; therefore the existence of the N type Ca channels as a separate entity has sometimes been questioned (Swandulla and Armstrong 1988).

Elucidation of the role of the three suggested calcium channels in skeletal muscle physiology requires further analysis of their kinetic and pharmacological properties.

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