Short communication

The Activation of Contraction and Extracellular Calcium in Striated Muscle of the Lamprey

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The slow voltage dependent calcium channels have been described in skeletal muscle fibres of adult frogs and rats (Sanchez and Stefani 1978; Donaldson and Beam 1983). Some data have suggested that they may participate in excitation-contraction coupling (ECC) (Beam et al. 1986; Ildefonse et al. 1985; Rios et al. 1986). However, some other investigations have shown the independence of activation of contraction on the slow calcium current (for a review see Caille et al. 1985). Our experiments were made on the suction muscle of lamprey, which is a typical striated twitch muscle (Samosudova et al. 1987). A characteristic feature of this muscle is its extrasynaptic sensitivity to acetylcholine (ACh) (Skorobovichuk and Itina 1968). Experiments were performed on segments of thin bundles of muscle fibres (80—120 μm in diameter), dissected from m. longitudinalis linguae of the lamprey (*Lampetra fluviatilis*). The membrane currents were recorded under voltage clamp conditions using the double sucrose gap method. Isotonic K_2SO_4 (K_2SO_4 80; TRIS 10) and isotonic $CaBr_2$ or $BaBr_2$ ($CaBr_2$ or $BaBr_2$ 80; TRIS 10) (in mmol/l) were used as the external solution. Both ends of the segments were immersed in internal solution containing either KCl (KCl 115; NaCl 5; EGTA 1; TRIS 10) or TEABr (TEABr 120; EGTA 1—5; TRIS 10). In other experiments the conventional microelectrode technique was used to record transmembrane potentials. The tension of muscle bundles was recorded using a force transducer (6MX2B, USSR). The solutions contained (in mmol/l): Ringer: NaCl 115; KCl 2.5; $CaCl_2$ 2; TRIS 10; the calcium free solution: NaCl 115; KCl 2.5; $MgCl_2$ 5; EGTA 2; TRIS 10; the sodium solution: sucrose 240; KCl 2.5; $CaCl_2$ 0—10; TRIS 10. Experiments were performed during the winter, at 18—20 °C.

Fig. 1 shows transmembrane ionic currents recorded in bundles of the lamprey muscle fibres in voltage clamp conditions. In Ringer solution, fast inward and delayed outward currents were observed in response to depolarizing pulse. The former could be blocked by tetrodotoxin (TTX, 10^{-7} mol/l), the latter by TEA^+ (30 mmol/l). In isotonic K_2SO_4, a hyperpolarizing pulse evoked an inward current, which did not decline at least during 30 ms. Obviously, these 3

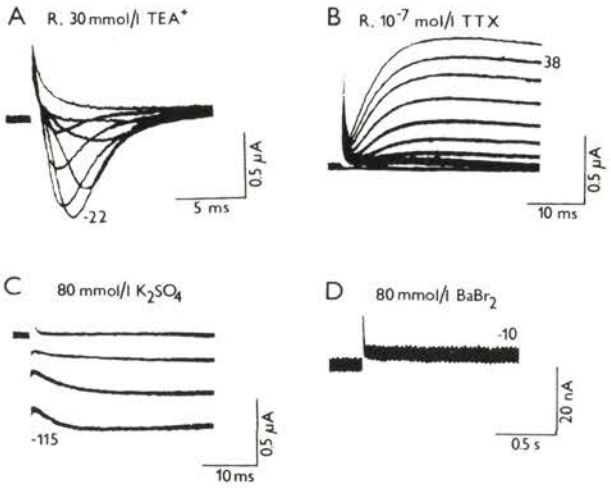

Fig. 1. Ionic currents recorded from a cut segment of a lamprey muscle fibres bundle. *A*, *B*: superimposed sodium and potassium currents in Ringer solution containing 30 mmol/l TEA^+ or 10^{-7} mol/l TTX respectively; Internal solution (mmol/l): KCl 115; NaCl 5; EGTA 1. *C*: inward rectifier current in isotonic K_2SO_4 as external and internal solutions. *D*: the absence of current carried by divalent ions in isotonic $BaBr_2$; Internal solution: TEABr 120; EGTA 2. Holding potential −100 mV (*A*, *B*, *C*) and 0 mV (*C*); pulse potentials are shown at the records (in mV).

current types can be identified as the fast sodium, the delayed potassium, and the inward rectifier current, as described in vertebrate skeletal muscle (Stefani and Chiarandini 1982). Inward currents carried by divalent cations, as observed in frog and rat muscle membranes (Sanchez and Stefani 1978; Donaldson and Beam 1983) could not be observed in any of the 24 bundles investigated (Fig. 1*D*). Calcium channels in striated muscles are known to be localized in T-tubular membranes (Stefani and Chiarandini 1982). In the lamprey muscle the T-tubular system is well developed (Samosudova et al. 1987). Consequently, the absence of functioning calcium channels is not due to the absence of T-membrane.

It has been suggested that activation of contraction in skeletal muscle fibres of amphibia and mammals does not require extracellular Ca^{2+} (Lüttgau and Spiecker 1979; Graf and Schatzmann 1984). On the other hand, the kinetics of the contraction-relaxation process may be markedly influenced by calcium removal from the external media. Namely, the plateau of potassium contractures becomes shorter, the force inactivation accelerates and the steady state potential dependence of force inactivation shifts to more negative potentials. Fig. 2*A* shows potassium contractures of lamprey muscle in normal Ca^{2+} solution and in Ca^{2+}-free solution containing EGTA (2 mmol/l) and Mg^{2+}

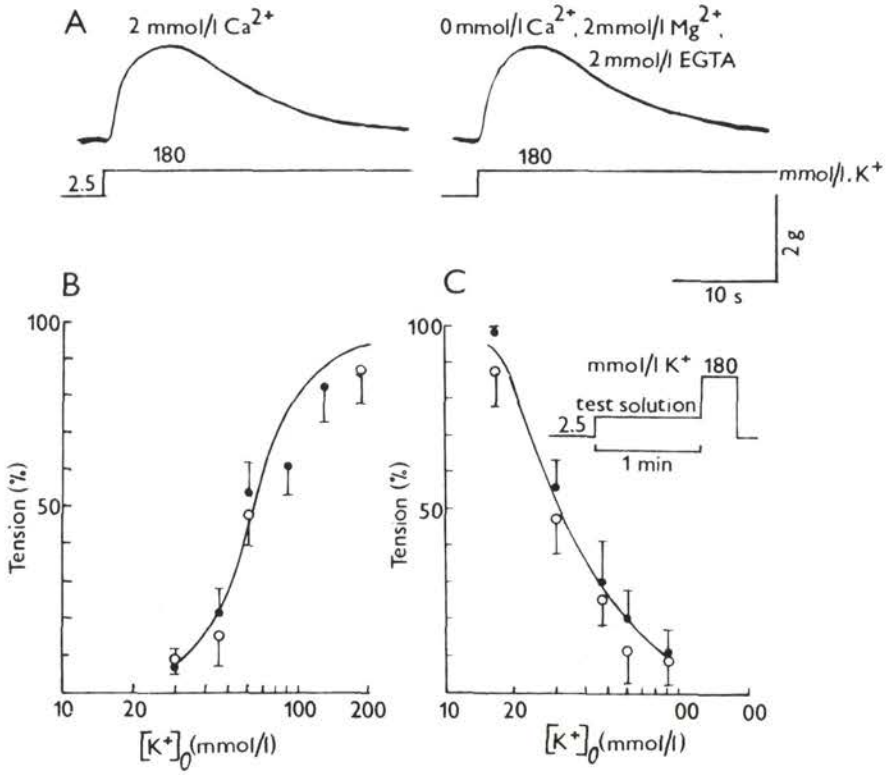

Fig. 2. Potassium contractures in a bundle of lamprey muscle fibres in normal and Ca^{2+}-free solution (*A*); activation (*B*) and inactivation (*C*) curves of potassium contractures in normal (2 mmol/l) Ca^{2+} concentration (●) and in Ca^{2+} and in Ca^{2+}-free solution (○). Tension in 180 mmol/l K^+ taken as 100 %. The points represent mean value of 3—10 bundles; standard errors of the mean are shown. The curves were drawn by eye.

(5 mmol/l). The contractures do not differ in amplitudes and time courses. Their mean half-time of decay was 11.9 ± 1.5 s ($n = 14$) and 11.6 ± 2.4 s ($n = 11$), respectively. Transmembrane potentials measured in these solutions were 77.2 ± 0.2 mV ($n = 10$) in normal and 76.0 ± 2.9 mV ($n = 7$) after 30—40 minutes in Ca^{2+}-free solution.

Calcium removal from external solution does not affect activation and/or inactivation curves of potassium contracture (Fig. 2*B*, *C*): the mean values are effectively the same for both normal and Ca^{2+}-free solution. It is interesting to note that the slope of the lamprey inactivation curve (identical for normal and Ca^{2+}-free solution) is similar to that obtained for pig muscle fibre bundles in Ca^{2+}-free solution by Graf and Schatzmann (1984). In the presence of calcium

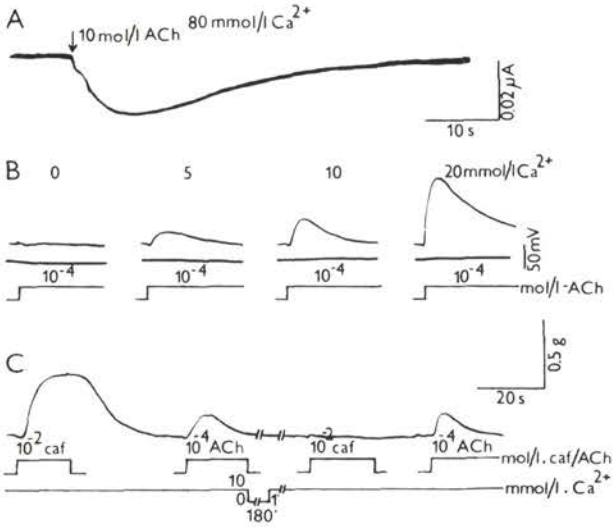

Fig. 3. Calcium current in response to ACh application recorded under voltage clamp from a cut segment of a bundle of lamprey muscle fibres in isotonic $CaBr_2$ (*A*); effect of external Ca^{2+} concentration on ACh contractures (upper trace) in the absence of essential membrane potential shift (lower trace) (*B*); effect of internal Ca^{2+} depletion (3 h washing in Ca^{2+}-free solution) on caffeine (caf) and ACh contractures. In *A* the integral solution contained 120 mmol/l TEABr and 2 mmol/l EGTA; holding potential −100 mV. *B* and *C*: in Na^+-free sucrose solution.

in external media the slope of the curve describing the inactivation process in the pig muscle is much steeper.

Our data suggest that the process of activation of contraction is independent of the presence of operative calcium channels, as no calcium currents can be detected in normally contracting lamprey muscle. This kind of channels may not be involved in ECC in muscles of other vertebrates as well. On the other hand, correlation between the absence of Ca-channels and that of changes in inactivation parameters in Ca^{2+}-free solution in the lamprey muscle points to the possibility that in higher vertebrates the calcium dependent changes in the inactivation process are associated with the functioning of calcium channels.

ACh is known to initiate Ca current through chemosensitive ionic channels in the synaptic membrane of frog muscle (Adams et al. 1980). In lamprey muscle the entire membrane is sensitive to ACh (Skorobovichuk and Itina 1968). In our experiments the inward Ca current was recorded in isotonic Ca^{2+} solution in response to ACh action (Fig. 3*A*). Also, we could show the dependence of ACh contracture on external calcium. In sodium free solution the contraction amplitude was a function of Ca^{2+} concentration in external media (Fig. 3*B*).

Moreover, in the absence of Na^+, ACh contractures developed without essential changes in the membrane potential. These data confirm our previous results which showed that in the lamprey muscle, the s.c. mechanism of "pharmaco-contraction coupling", i.e. the ability of a chemical transmitter to mediate contraction without depolarization is also operative in parallel with the excitation-contraction coupling mechanism (Skorobovichuk and Nasledov 1978).

External Ca^{2+} entering the fibres through ACh-activated channels is believed to induce force development either directly or by the mechanism of "Ca-induced Ca release". In our experiments on the lamprey muscle caffeine-induced and ACh-induced contractures were recorded (Fig. 3*C*). It appeared that after washing the muscle in Ca^{2+}-free Ringer for 3 h, caffeine could not induce any tension, however the ACh contracture still developed with an approximately normal amplitude. Obviously, depletion of the intracellular Ca^{2+} stores did not prevent ACh induced contraction activated by Ca^{2+} entered from the outside. This suggests a direct participation of external Ca^{2+} in force activation.

Thus, the lamprey muscle can be considered a natural model of the indepencence of depolarization evoked contraction of voltage dependent calcium channels in the surface membrane; it also illustrates the possibility of utilization of external Ca^{2+} for ACh induced contractile activation.

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Final version accepted February 26, 1988