

Decoupling of the Excitation-Contraction Link in Strontium Solutions by Cooling in Crayfish Muscle Fibres

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Abstract. The excitation-contraction (E-C) coupling in crayfish muscle fibres can be dissociated at low temperatures (5°C–3°C) if Sr ions are substituted for Ca ions. The decoupling becomes irreversible if the fibre was exposed to low temperatures and strontium salines for more than 45 min. The dissociation depends on the $\text{Ca}^{2+}/\text{Sr}^{2+}$ ratio in the extracellular medium. The potentiating effect of Ca^{2+} on E-C coupling dominates over the inhibitory action of Sr^{2+} at Ca/Sr around 0.3. The dissociation of the strontium action potential from the contraction is also weakened by increasing the intracellular Ca^{2+} concentration, $[\text{Ca}]_i$, with subthreshold concentrations of caffeine (0.2 mmol/l). Procaine action potentials initiated by low concentrations of procaine (2 mmol/l) in the presence of Ca^{2+} are not dissociated. The dissociation appearing at higher procaine concentrations may be explained by the action of procaine on the sarcoplasmic reticulum and may be abolished by subthreshold caffeine doses, i.e. by increasing $[\text{Ca}]_i$. Action potentials induced by the blockade of K channels with TEA^+ (tetraethylammonium) in Ca^{2+} solutions cannot be dissociated from contraction by cooling. The results show that in this kind of dissociation a decisive role is played by strontium ions which enter the cell through the calcium channel. Sr^{2+} are supposed to be less effective in activating contraction as compared with Ca^{2+} ; this difference becomes accentuated at low temperatures.

Key words: Excitation-contraction coupling — Decoupling by cooling — Strontium ions — Potentiators of electrogenesis — Muscle fibres — *Astacus fluviatilis*

Introduction

We have shown recently that the strontium action potential in single muscle fibres of the crayfish can be dissociated from the contraction when the temperature of the fibre is decreased to about 5°C (Lipskaja and Zacharová 1977). The rate of recovery of the excitation-contraction link (EC link) from dissociation depends on

the time of exposure to the decoupling temperature. The block of the EC link is specific for the strontium action potential and is absent if the active membrane responses are generated in the presence of calcium ions.

The 'cooling' procedure for blocking the contractile machinery has evident advantages over those which were used in the past for blocking contraction in crustacean muscle fibres (Zachar 1971; Suarez-Kurtz and Sorenson 1977; Brulé et al. 1977). The new method has successfully been used recently to perform measurements of passive and active membrane properties in crayfish muscle fibres under conditions of abolished muscle tension generation (Poledna et al. 1979, 1980). The method could be exploited still more, if the mechanism of the E-C decoupling at low temperatures was known in detail. The aim of the experiments reported in this paper was to find out, which part of the E-C coupling process is mostly responsible for the failure of the strontium action potential to activate the contractile apparatus in single muscle fibres of the crayfish. For this purpose we compared the effect of cooling on the activation of contraction initiated by non-strontium action potentials, namely by procaine spikes (Fatt and Katz 1953; Ozaki et al. 1966) and TEA⁺ spikes (Fatt and Ginsborg 1958), respectively. The ability of the contractile apparatus to respond to direct activation at low temperatures was checked by caffeine, which is known to mobilize calcium from the internal membrane systems of the crayfish (Uhrík and Zacharová 1968, 1976).

The results were published in brief elsewhere (Lipskaja and Zacharová 1977, 1980).

Material and Methods

The experiments were performed on single muscle fibres isolated from m. extensor carpopoditi of the crayfish *Astacus fluviatilis*. The average diameter of the fibres was $410 \pm 10 \mu\text{m}$ ($\pm$ SEM); the average slack length of the fibres was $l_0 = 10 \text{ mm}$. The fibre was fixed in the experimental chamber and stretched to $1.2 l_0$. The experimental chamber enabled a rapid exchange of the bathing medium. Stimulation of the fibre and recording of the electrical membrane responses was performed by means of microelectrodes; isometric tension was recorded by the RCA 5734 transducer (Zachar and Zacharová 1966). The recording microelectrodes were filled with 3 mol/l KCl, the stimulating ones with 2 mol/l K-citrate. The pulse duration was 10–60 ms.

The normal crayfish saline (van Harreveld solution) had the following composition (in mmol/l): Na⁺ 208.4; K⁺ 5.4; Ca²⁺ 13.5; Mg²⁺ 5.6; Cl⁻ 241; TRIS⁺ 2.0; pH = 7.4. In 'strontium' saline Sr²⁺ ions (27 mmol/l) were substituted for the Ca²⁺ ions. Procaine was used in concentration 2–8 mmol/l, caffeine in concentration 0.2 mmol/l and TEA⁺ in concentration 50 and 70 mmol/l. The osmolarity of solutions was maintained constant by equivalent changes in the concentration of Na⁺ ions.

The temperature of solution in the bath was maintained at the required level by means of a thermistor-controlled cooling system as described elsewhere (Gulišek 1975).

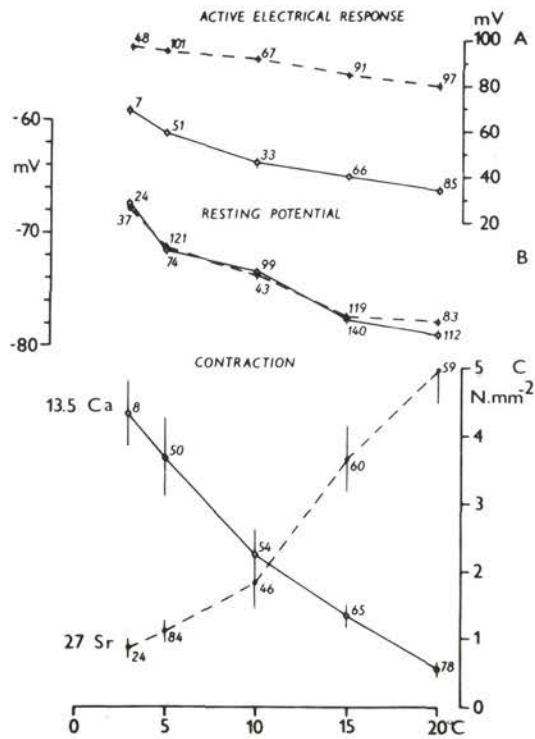


Fig. 1. Temperature-dependent changes in electrical and contractile parameters of isolated muscle fibres of the crayfish *Astacus fluviatilis*. Full lines and (○): in the presence of Ca ions; dashed lines and (●): Ca ions replaced by Sr ions. A — active electrical membrane responses; B — resting membrane potential; C — isometric twitch tension. Numbers over the average values ($\pm$ SEM) shows the number of measurements.

Results

Influence of temperature on the excitation-contraction coupling in the presence of Ca or Sr ions

As shown in Fig. 1 the presence of Sr ions is unavoidable for the dissociation of the E-C coupling to occur in crayfish muscle fibres at low temperatures. While the active membrane response (A) and the resting membrane potential (B) change with temperature in accordance with each other, an opposite change in the amplitude of contraction (C) with temperature is observed in the presence of Ca and Sr ions; in the presence of Ca ions the contraction amplitude grows with the decrease of temperature, while decreasing in the presence of Sr ions. Average

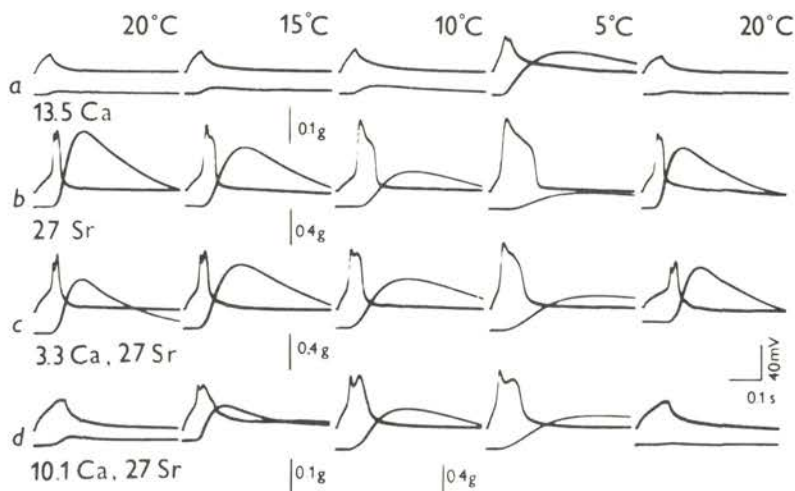


Fig. 2. The effect of calcium ions on the excitation-contraction coupling initiated by the strontium action potential at different temperatures. Active membrane responses (upper trace) and twitch tension (lower trace) recorded at indicated temperatures *a*: in the presence of Ca ions (13.5 mmol/l); *b*: in the presence of Sr ions (27 mmol/l); *c*: as in *b* but after addition of Ca ions (3.4 mmol/l). Note change in gain when recording tension. *d*: as in *c*, but after raising the Ca concentration (10.1 mmol/l).

values of the above parameters were obtained from records such as those demonstrated in Fig. 2 (first two rows; *a*, *b*). The first row (*a*) demonstrates changes in the active membrane response (upper trace) and the twitch (lower trace) recorded in normal van Harreveld solution containing 13.5 mmol/l of Ca ions. The records in the second row (*b*) illustrate changes in the active electrical response and the twitch in a solution containing Sr ions (27 mmol/l) instead of Ca ions. Replacing Ca ions by Sr ions results in a propagated strontium action potential at temperatures as high as 20°C (Fatt and Ginsborg 1958), resulting in a powerful twitch (Zacharová et al. 1962). With the decreasing temperature, the strontium action potential becomes prolonged, its amplitude raises, and a distinct plateau occurs; on the other hand, the twitch becomes weaker. This effect is fully reversible (last record in *b*; at 20°C) if the fibre was exposed to 'dissociation condition' over a shorter time interval (approx. 30–45 min).

Reversibility of dissociation of the strontium action potential from contraction

If the muscle fibre of the crayfish is exposed to strontium saline at low temperature for more than 45 min, it may remain dissociated also after rewarming. We termed this dissociation as irreversible or partially reversible dissociation (Lipskaja and

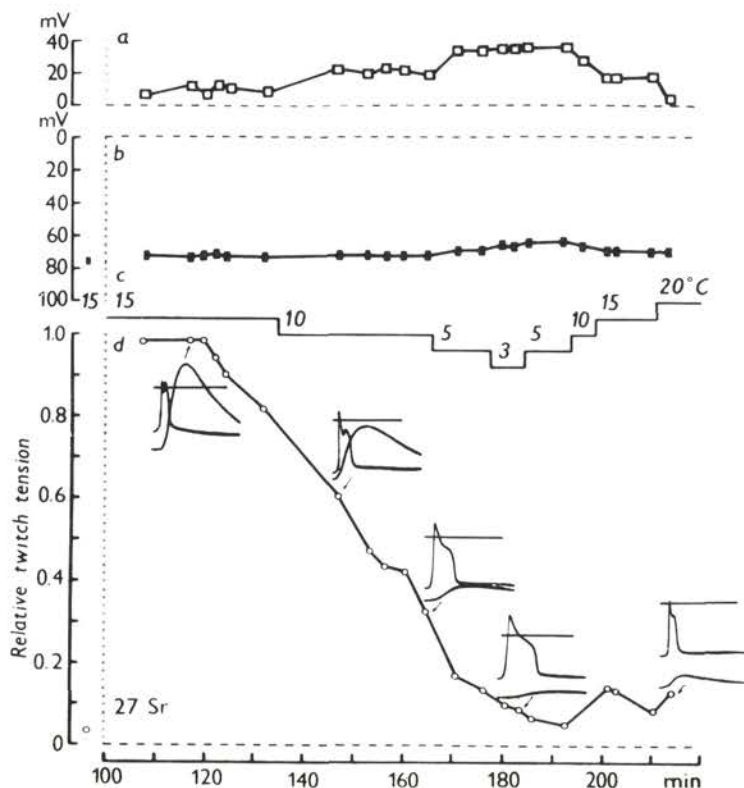


Fig. 3. Dissociation of the strontium action potential from contraction after long lasting exposure of an isolated fibre to strontium saline (27 mmol/l) and low temperatures (as indicated in *c*). *a*: overshoot of the strontium action potential; *b*: resting membrane potential; *c*: temperature of the fibre bath; *d*: relative twitch tension. Inset figures: simultaneous records of the strontium action potential and isometric contraction at indicated times. Fibre 60 D; $l_0 = 9.0$ mm, diameter: $376.3 \mu\text{m}$

Zacharová 1977) in order to differentiate it from the reversible dissociation, which can be induced by short exposures ($t < 45$ min) of fibres to strontium saline at low temperatures. The duration of after-dissociation is proportional to the drop in temperature, the time of exposure to the Sr^{2+} saline and the concentration of Sr^{2+} . Fig. 3 demonstrates this after-effect in a fibre which was kept in 27 mmol/l Sr^{2+} saline at room temperature for 100 min and subsequently exposed in steps to 3°C. After about 100 min exposure to cold strontium saline the temperature of the bath was brought back to 20°C. The recovery of tension upon rewarming was only partial; the strontium action potential parameters recovered, however, to normal values. The irreversible dissociation thus represents a method of choice how to

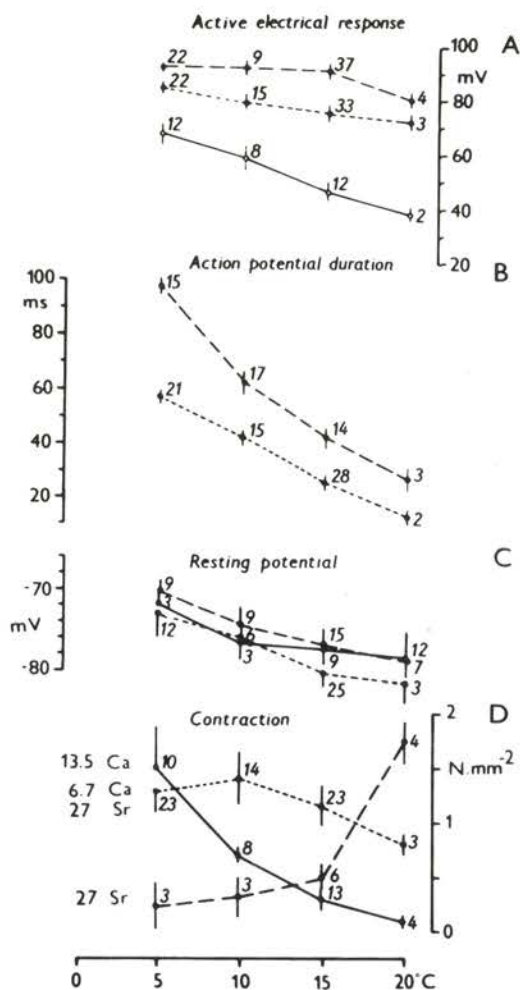


Fig. 4. Temperature-dependent changes in electrical membrane parameters and contraction in the presence of Ca ions (full line), Sr ions (dashed line — long dashes), and after the addition of Ca (6.7 mmol/l) to the strontium solution (short dashes).

A — active electrical membrane responses, B — duration of the action potential, C — membrane potential, D — isometric contraction tension. Numbers over the average values ($\pm$ SEM) show the number of experiments.

keep the contractile apparatus unresponsive to the strontium action potential in the muscle membrane.

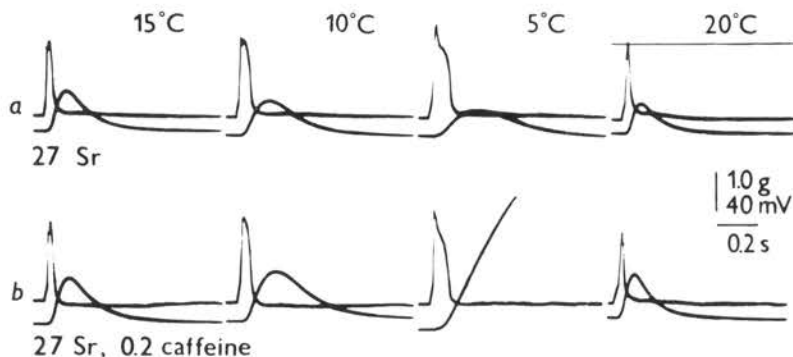


Fig. 5. The effect of a subthreshold concentration of caffeine on cold-induced dissociation of the E-C coupling in the presence of Sr ions. *a*: changes in strontium action potential (upper trace) and contraction (lower trace) with decreasing temperature. *b*: as in *a* but after the addition of caffeine (0.2 mmol/l).

Influence of Ca ions on the dissociation of the E-C link by cooling

Cold dissociation of the E-C coupling in the presence of Sr ions becomes markedly modified when Ca ions are added to the strontium solution. As illustrated in Fig. 2c the addition of as many as 3.3 mmol/l of Ca ions (one fourth of the physiological concentration) results in a pronounced inhibition of the cold dissociation effect at the Sr ion concentration remaining unchanged. The contraction amplitude is higher (note change in calibration) despite the smaller and shorter action potential. When the concentration of Ca ions is raised to 10.1 mmol/l (Fig. 2d) the E-C dissociation disappears almost entirely, the records resemble those of the responses in the presence of Ca ions alone. Fig. 4 enables to compare the changes in the electrical membrane parameters and in the muscle fibre contraction respectively with temperature both either in the presence of Ca ions (full line) or Sr ions alone (dashed line); as well as following the addition of 6.7 mmol/l of Ca ions to the strontium solution (short-dashed line). The twitch potentiation following the addition of Ca ions (Fig. 2c and Fig. 2d) may be explained on the assumption that the Ca ions entering the cell during the action potential are more effective in activating the contraction than are the Sr ions. This suggestion is supported by the experiment illustrated in Fig. 5. The addition of caffeine in a subthreshold concentration (0.2 mmol/l; Zacharová and Zachar 1968) to the strontium solution (Fig. 5b) results rather in strengthening of the contraction with the decrease in temperature than in inhibition (compare records in Fig. 5a and Fig. 5b). The effect is reversible.

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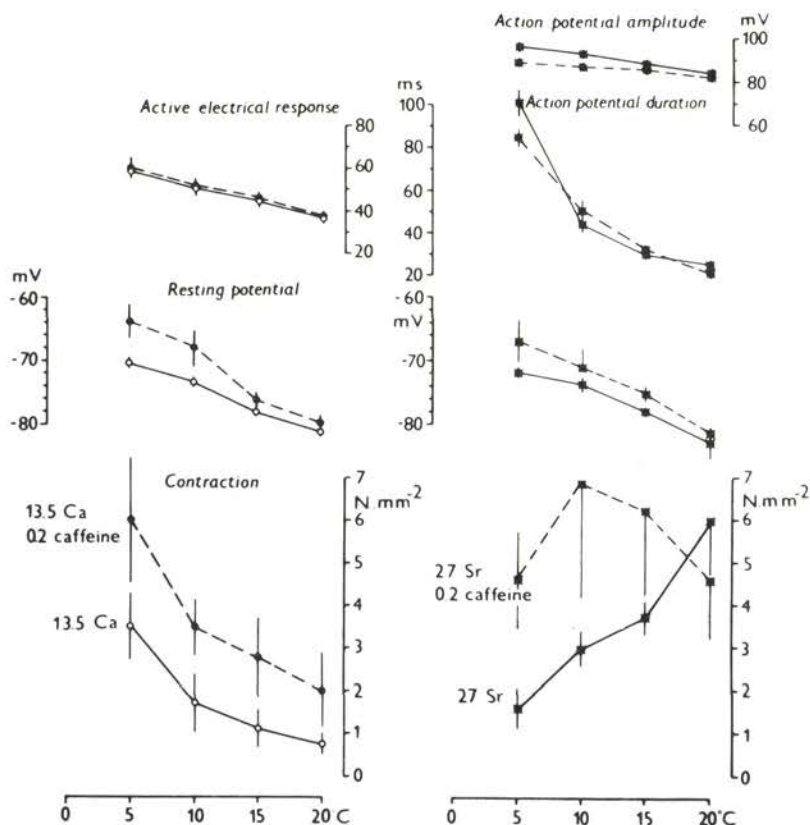


Fig. 6. The effect of caffeine on electrical and contractile parameters of crayfish muscle fibres. *Left column:* in the presence of Ca ions (13.5 mmol/l). *Right column:* in the presence of Sr ions (27 mmol/l). Dashed line-connected full symbols indicate mean values obtained after the addition of caffeine.

reticulum in the presence of low caffeine concentrations, resulting in a more pronounced contractile effect of external Sr ions.

Fig. 6 summarizes the effects of subthreshold caffeine concentrations on basic electrical and contractile parameters of the crayfish muscle fibre in the presence of Ca ions (left) and in the presence of Sr ions (right). The potentiation of the twitch amplitude by caffeine in calcium salines is added to the facilitatory effect of cooling, with the result that the twitch — temperature relations change in parallel. The situation is different in the strontium saline, where the caffeine effect is counteracted by the decreasing efficacy of strontium ions to induce contraction with the decrease in temperature. As result a bell-shaped twitch-temperature relation is observed with maximum at about 10°C.

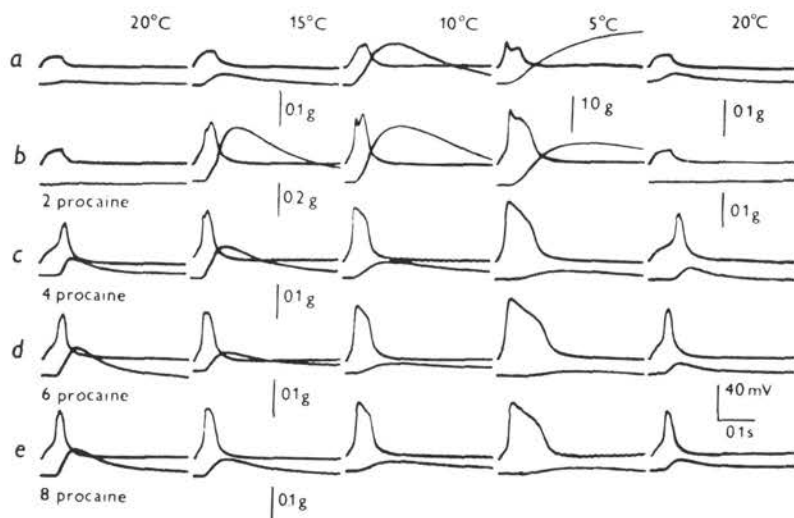


Fig. 7. Temperature dependence of the excitation-contraction coupling initiated by the procaine action potential (*b–e*). *a*: active membrane responses (upper trace) and isometric contractions (lower trace) in the control procaine-free crayfish saline. The numbers in *b–e* indicate concentration of procaine in mmol/l in the crayfish saline.

E–C coupling in the presence of procaine

In the presence of procaine, the gradual electrogenesis of crayfish muscle fibres changes to an “all-or-nothing” type (for review see Zachar 1971), as also documented by the records shown in Fig. 7. With the decrease in temperature the procaine action potentials increase and broaden similarly as do the strontium action potentials.

At 20°C the procaine action potential is accompanied by a twitch the amplitude of which increases with the amplitude of the action potential. The latter increases in turn with the increasing concentration of procaine (Fig. 7*b–e*; 2–8 mmol/l). Despite an increase in the procaine action potential with the decreasing temperature the contraction strength decreases, and eventually E–C decoupling occurs; low procaine concentration (2 mmol/l) represents the only exception. This effect can be explained assuming that procaine can inhibit Ca ion release from the sarcoplasmic reticulum (Uhrík and Zacharová 1976; see also Zachar 1971). At procaine concentrations exceeding 4 mmol/l (Fig. 7*c–e*) this effect obviously prevails over the potentiation effect of procaine on the electrogenesis in the superficial membrane. The latter effect prevails, however, at low procaine concentrations (2 mmol/l) resulting in the strengthening of the contraction with the increasing temperature (Fig. 7*b*). The inhibitory effect of procaine on

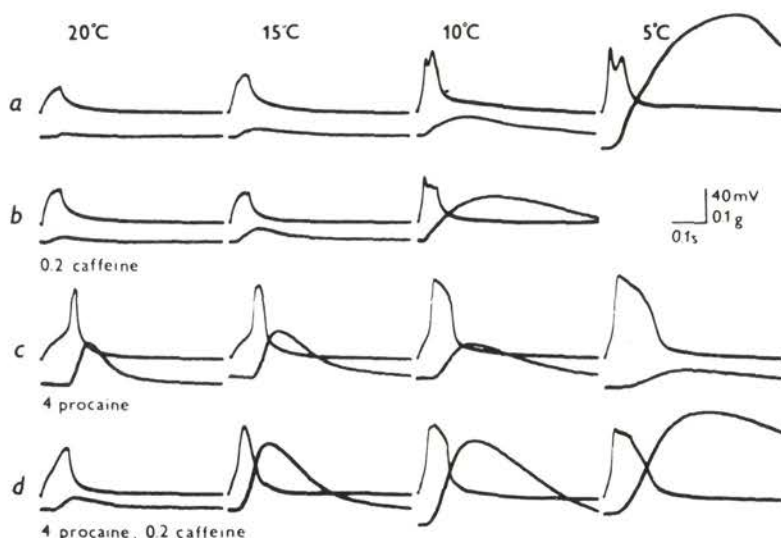


Fig. 8. The effect of caffeine on the procaine action potential and the associated twitch. Upper traces: active membrane responses; lower traces: isometric contractions. *a*: control records in the crayfish saline; *b*: after the addition of caffeine (0.2 mmol/l); *c*: after the addition of procaine (4 mmol/l); *d*: as in *c* but following the addition of caffeine (0.2 mmol/l).

the sarcoplasmic reticulum can be counteracted by subthreshold concentrations of caffeine (0.2 mmol/l in Fig. 8d).

E-C coupling in the presence of TEA⁺ ions

Since procaine interferes with the events both in the superficial membrane and the membranes of the sarcoplasmic reticulum the suggestion concerning the primary role of Sr ions in cold dissociation had to be tested using such an agent which would lead to conversion of gradual electrogenesis in the superficial membrane to the "all-or-nothing" type, leaving other links of the E-C coupling unaltered.

Aimed at this, temperature dependence of the E-C coupling initiated by the action potential generated in the presence of tetraethylammonium ions (TEA⁺) was tested. These ions are able to block potassium channels in the crayfish muscle membrane (Henček et al. 1978) resulting in the initiation of TEA action potentials, as also demonstrated in Fig. 9b, c (50 and 70 mmol/l). The twitch amplitude rises with the amplitude of the action potential, due either to a higher TEA⁺ concentration or to the decreased temperature. All experiments were performed in the presence of Ca ions. The absence of dissociation of contraction from the action

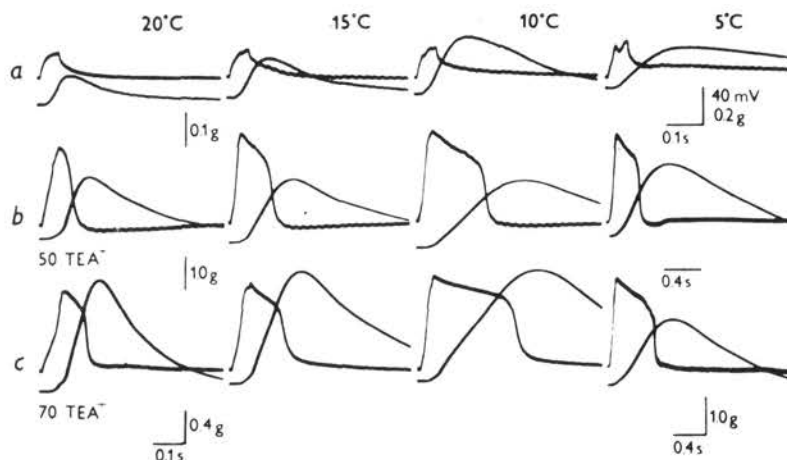


Fig. 9. Temperature dependence of the E-C coupling in a crayfish muscle fibre in the presence of TEA⁺. Upper traces: active membrane responses; lower traces: isometric contractions. *a*: control records in the crayfish saline, *b*: after the addition of TEA⁺ (50 mmol/l). Occurrence of TEA action potentials. *c*: as in *b*, but the TEA⁺ concentration was raised (70 mmol/l).

potential clearly documents that the change in the active membrane response is not a determining factor in the decoupling by cooling.

Discussion

The present experiments were aimed at establishing the share of electrogenic and or contractile effects of strontium ions in the dissociation of the E-C coupling by cooling (Lipskaja and Zacharová 1977). From the results presented herein it becomes obvious that it is the intracellular effect of strontium ions on the activation of the contractile apparatus that is decisive in the mechanism of cold-induced dissociation.

The possible electrogenic action of Sr ions could be ruled out in experiments in which procaine and tetraethylammonium ions (TEA⁺) were used to induce the propagated action potential in the superficial membrane of the muscle cell. Procaine and TEA are drugs that can potentiate the action potential of the calcium type by inhibiting the potassium conductances (Hagiwara et al. 1969; Henček et al. 1978; Zacharová et al. 1980). Activation of potassium outward ionic currents interferes with the calcium inward current (Zachar and Henček 1979); due to this, gradual responses appear in the crayfish muscle membrane in normal ionic environment. Suppression of membrane potassium conductances results then in the occurrence of propagated action potentials of the "all-or-nothing" type. These are calcium action potentials since the inward current is carried by calcium ions.

Contrary to strontium action potentials, the calcium action potential cannot be dissociated from contraction at low temperatures. The observed dissociation of the procaine-induced action potential and contraction may be explained by inhibition of the Ca release from the sarcoplasmic reticulum by the respective procaine concentration.

The strontium ion thus plays a decisive role in the cold dissociation. During the strontium action potential Sr^{2+} penetrate the cell via the membrane calcium channel which is at least as permeable to Ca ions as to Sr ions (Zachar and Henček 1979). The occurrence of the action potential in the strontium environment is also partly due to a block of the membrane K conductances by Sr ions.

Since identical amounts of divalent ions can be assumed to permeate the cell membrane during both calcium and strontium action potential, the cold dissociation may be suggested to be associated with a lower efficacy of Sr ions as the contractile apparatus activators; this becomes more pronounced at low temperatures.

This hypothesis has also been supported by experiments in which Ca ions were added to the strontium solution, resulting in the abolishment of E-C decoupling. The experiments using caffeine showed that this effect was associated with the intracellular action of Ca ions added to the extracellular fluid and entering the cell due to activation of the membrane Ca channels.

A lower contractile effectiveness of Sr ions as compared with Ca ions has also been reported in the literature. It is known that Ca ions cannot substitute for Sr ions in the electromechanical coupling as tested by potassium depolarisation (Zacharová and Zachar 1971; Boručuk and Zacharová 1973). In experiments using electrophoretic injection of Ca^{2+} , Sr^{2+} and Ba^{2+} Matsumura and Mashima (1976), Matsumura (1981) observed that the threshold level of Sr^{2+} required to produce contraction is about twice higher than that of Ca^{2+} . The injected Ca^{2+} and Sr^{2+} acted additively in the contraction if the amounts of the injected ions were small.

The sensitivity to Sr^{2+} and Ca^{2+} in mechanically skinned skeletal muscle fibres of three crustaceans (barnacle, crayfish and crab) has been recently investigated by Stephenson and Williams (1980). The maximum force response induced by Sr^{2+} was only 0–10% of that induced by Ca^{2+} in fibres denuded shortly after the dissection. These authors also obtained evidence that the Sr ions can bind to all Ca^{2+} regulatory sites, being, however, able to 'turn on' only a small fraction of them.

The lower effectiveness of Sr ions in comparison to Ca ions on biochemical contractile systems (lower affinity to troponin, lower uptake by the sarcoplasmic reticulum) seems to be their general property (Weber et al. 1966, Ebashi et al. 1969, Kerrick et al. 1980). A lower uptake of Sr^{2+} by the sarcoplasmic reticulum vesicles of the crayfish muscle fibres has recently been reported by Tomková and Kontšeková (1980).

Contractility of myofibrills is known to be reduced with the decrease in temperature as shown in experiments using glycerinated fibres or isolated actomyosin systems in vitro (Weber and Portzehl 1954; Fuchs et al. 1975). Thus, activation of contraction may be absent at lower temperatures, due to the lower effectiveness of Sr ions.

References

- Boručuk B., Zacharová D. (1973): Different effect of strontium ions on potassium and caffeine contractures in crayfish muscle fibres. *Physiol. Bohemoslov.* **22**, 57
- Brulé G., Guilbault P., Haudecoeur G. (1977): Effects de l'hypertonie sur le couplage excitation-contraction de la fibre musculaire squelettique de crabe. I (Aspects électrique mécanique). *J. Physiol. (Paris)*, **73**, 663—674
- Ebashi S., Endo M., Ohtsuki I. (1969): Control of muscle contraction. *Quart. Rev. Biophys.* **4**, 351—384
- 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
- Fatt P., Katz B. (1953): The electrical properties of crustacean muscle fibres. *J. Physiol. (London)*, **120**, 171—204
- Fuchs F., Hartshorne D. J., Barns E. M. (1975): ATPase activity and superprecipitation of skeletal muscle. Actomyosin of frog and rabbit: Effect of temperature on calcium sensitivity. *Comp. Biochem. Physiol.* **51**, 165—170
- Gulišek D. (1975): A temperature clamp device for isolated cells. *Physiol. Bohemoslov.* **24**, 440
- Hagiwara S., Hayashi H., Takahashi K. (1969): Calcium and potassium currents of the membrane of a barnacle muscle fibre in relation to the calcium spike. *J. Physiol. (London)*, **205**, 115—129
- Henček M., Zachar J., Zacharová D. (1978): Membrane currents in a calcium type muscle membrane under voltage clamp. *Physiol. Bohemoslov.* **27**, 457—466
- Kerrick W. G. L., Malencik D. A., Hoar P. E., Potter J. D., Coby R. L., Pocinwong S., Fisher E. (1980): Ca^{2+} and Sr^{2+} activation: comparison of cardiac and skeletal muscle contraction models. *Pflügers Arch.* **386**, 207—213
- Lipskaja E., Zacharová D. (1977): Conditions for dissociation of the excitation-contraction link in crayfish muscle fibres at low temperatures. *Physiol. Bohemoslov.* **26**, 457—458
- Lipskaja E., Zacharová D. (1980): The effect of strontium and calcium ions on the excitation-contraction link at low temperatures. *Physiol. Bohemoslov.* **29**, 454
- Matsumura M. (1981): Strontium as the substitute for calcium in the excitation-contraction coupling of crayfish muscle fibers. In: *Handbook of Stable Strontium* (Ed. S. C. Skoryna), pp. 309—319, Plenum Publishing Corporation, New York
- Matsumura M., Mashima H. (1976): Contraction produced by intracellular injection of calcium, strontium and barium in the single crayfish muscle fibers. *Jan. J. Physiol.* **20**, 145—157
- Ozeki M., Freeman A. R., Grundfest H. (1966): The membrane components of crustacean neuromuscular systems. I. Immunity of different electrogenic components to tetrodotoxin and saxitoxin. *J. Gen. Physiol.* **49**, 1319—1334
- Poledna J., Zacharová D., Lipskaja E. (1980): Temperature coefficients of membrane parameters in muscle fibres of the crayfish. *Abstr. Papers XXVIII. Int. IUPS Congress No 2808*, vol. **14**, 644
- Poledna J., Zacharová D., Lipskaja E., Zachar J. (1979): Contribution of the T-system to the membrane capacity determined from the foot of the action potential. *Physiol. Bohemoslov.* **2**, 577—580

- Stephenson D. G., Williams D. A. (1980): Activation of skinned anthropod muscle fibres by Ca^{2+} and Sr^{2+} . *J. Muscle Res. Cell Motility* **1**, 73—87
- Suarez-Kurtz G., Sorenson A. L. (1977): Inhibition by hypertonic solutions of Ca-dependent electrogenesis in single crab muscle fibers. *J. Gen. Physiol.* **70**, 491—505
- Tomková Ž., Kontšeková M. (1980): The uptake of strontium ions by the sarcoplasmic reticulum of the crayfish. *Physiol. Bohemoslov.* **29**, 472—473
- Uhrík B., Zacharová D. (1968): Ultrastructural changes in the internal membraneous system of crustacean muscle fibres during caffeine contracture. *Physiol. Bohemoslov.* **17**, 496
- Uhrík B., Zacharová D. (1976): Recovery of ultrastructural changes accompanying caffeine contractures in isolated muscle fibres of the crayfish. *Pflügers Arch.* **364**, 183—190
- Weber A., Herz R., Reiss I. (1966): Study of the kinetics of calcium transport by isolated fragmented sarcoplasmic reticulum. *Biochem. Z.* **345**, 329—369
- Weber, H. H., Portzehl M. (1954): The transference of the muscle energy in the contraction cycle. *Progr. Biophys.* **4**, 60—111
- Zachar J. (1971): *Electrogenesis and Contractility in Skeletal Muscle Cells*. University Park Press, Baltimore and London; Publishing House of the Slovak Academy of Sciences, Bratislava
- Zachar J., Henček M. (1979): Excitable ion channels in a calcium type muscle membrane of the crayfish. *Brain Research Bulletin* **4**, 199—201
- Zachar J., Zacharová D. (1966): Potassium contractures in single muscle fibres of the Crayfish. *J. Physiol. (London)*, **186**, 598—618
- Zacharová D., Zachar J. (1968): Caffeine contractures and their dependence on the bound and extracellular calcium in isolated muscle fibres of the crayfish. *Čs. Fyziol.* **17**, 47 (in Slovak).
- Zacharová D., Zachar J. (1971): The effect of some divalent cations on the electro-mechanical coupling in isolated muscle fibres of the crayfish. *Fiziol. Zh. SSSR*, **57**, 1331—1339 (in Russian).
- Zacharová D., Zachar J., Henček M. (1962): The influence of strontium ions on the excitation and contraction of the single crustacean muscle fibre. *Abstr. Commun. XXIIth Physiol. Congress*, 905
- Zacharová D., Lipskaja E., Henček M. (1980): The effect of procaine on ionic currents and excitation-contraction coupling in muscle fibres of the crayfish. *Abstr. papers XXVIII. Int. IUPS Congress*, No 3717 vol **14**, 796

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