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Effect of Divalent Cations on the Potassium Contracture of Slow Muscle Fibers of *Rana temporaria*

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Summary. K contractures of single slow muscle fibers of *Rana temporaria* were measured isometrically in the presence of normal, reduced, and increased Ca^{2+} concentrations at 18 to 20°C. At normal Ca^{2+} concentration (1.8 mM) contracture tension decreased from its peak value of 35.4 ± 8.2 N/cm² to 59.4 ± 23.9% within one minute, and to 48.3 ± 27% within two minutes (30 fibers). Peak tension was virtually unaffected by changes of the Ca^{2+} concentration, but maintenance of tension was impaired by low (0.2 mM), and improved by high (10 mM) Ca^{2+} concentrations. When Ca^{2+} was added during the K contracture, there was practically no restoration of lost tension. Effects similar to those of Ca^{2+} were observed upon addition of foreign divalent cations to the medium. Co^{2+}, Ni^{2+}, and Cd^{2+} were slightly more effective than Ca^{2+} and Mn^{2+}; the smallest effects were obtained with Mg^{2+}, Sr^{2+}, and Ba^{2+}. The effects of foreign divalent cations were independent of the presence of Ca^{2+}. It is concluded that in slow fibers of *Rana temporaria* maintenance of contracture tension is not due to an influx of Ca^{2+} ions. Instead, binding of divalent cations to superficial sites seems to be essential.

Key Words slow muscle fiber · K contracture · divalent cations

Introduction

Slow muscle fibers of the frog are known to maintain long lasting mechanical tension during continued depolarization of their membrane (Kuffler & Vaughan Williams, 1953; Lüttgau, 1963; Nasledov, Zachar & Zacharova, 1966; Lännergren, 1967; Elul, Miledi & Stefani, 1970). There is general agreement that the contractile system is activated by release of Ca^{2+} from intracellular stores; external Ca^{2+} is obviously not essential during the early phase of tension development (Miledi, Parker & Schalow, 1977, 1981). In contrast, extracellular Ca^{2+} is important during the maintained phase of contracture in slow fibers of the chicken (Page, 1969; Huerta & Stefani, 1981; Kikuchi & Schmidt, 1983), *Xenopus laevis* (Lännergren, 1967) and *Rana pipiens* (Huerta, Muñiz & Stefani, 1986). Foreign cations such as Ni^{2+} or Co^{2+} cannot replace Ca^{2+} in their effect on maintained tension of slow fibers of the chicken (Kikuchi & Schmidt, 1983) and *Rana pipiens* (Huerta et al., 1986).

In slow fibers of *Rana temporaria* the situation is less clear. No influence of external Ca^{2+} on maintained tension was found by Lüttgau (1963) and Gilly and Hui (1980), while Schaechtelin (1961) and Nasledov et al. (1966) found a small effect. A marked effect was described by Pauschinger and Brecht (1961) after prolonged soaking of fiber bundles in low Ca^{2+} solution, and Miledi et al. (1977, 1981) showed in the same species that intracellular calcium signals during maintained depolarization became more transient with lowering of the extracellular Ca^{2+} concentration.

In view of these discrepancies it seemed necessary to reexamine—in *Rana temporaria*—the effect of external Ca^{2+} on the ability of slow fibers to maintain tension. Furthermore, it was important to investigate whether foreign divalent cations exert similar or opposite effects. The results of these experiments support the view that in slow fibers of *Rana temporaria* maintenance of tension is not due to a continuous influx of Ca^{2+} ions; instead, binding of divalent cations to sites on the external surface of the slow fiber membrane seems to control the intracellular Ca^{2+} activity during prolonged depolarization. Some of these results have been published in preliminary form (Schmidt, 1987).

Materials and Methods

Slow fibers were dissected from iliofibularis and cruralis muscles of *Rana temporaria*[1] according to the selection procedure described previously (Lüttgau, 1963; Lännergren, 1967). They were mounted in the narrow groove of a perfusion chamber.

[1] With permission of the Government of the Saarland (§ 28 Abs. 3 No. 3 SNG).

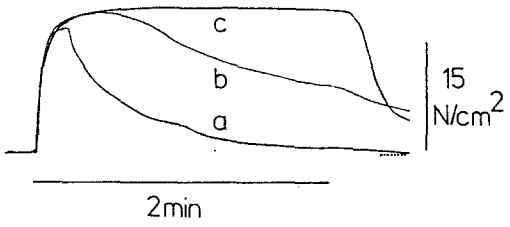

Fig. 1. Influence of external Ca^{2+} concentration on the time course of K contracture. A slow fiber was equilibrated with Ringer's containing 0.2 (*a*), 1.8 (*b*) and 10 mM Ca^{2+} (*c*) for 2 min each before 95 mM K-Ringer's containing the same Ca^{2+} concentrations was applied. Note increased rate of relaxation in 0.2 mM Ca^{2+}, and no relaxation at all in 10 mM Ca^{2+}. In this and the following Figures the time scales below records indicate periods of 95 mM K application. Intervals between records, 30 min; temperature, 18°C. Records were superimposed photographically. Diameter of fiber, 70 μm

Solutions entered and left the chamber through plastic tubes which were opened and closed by valves operated automatically; thereby solutions could be changed in a highly reproducible manner, and in quick succession. Contractures were measured isometrically (Lehmann & Schmidt, 1979), and the results are expressed as mean ± standard deviation. The resting length of the fibers was set at slightly more than slack length, the applied tension was 0.5 to 1 N/cm². Shortening during maximum contractures was less than 3% of the fiber length.

Solutions

Normal Ringer's had the following composition (mM): KCl 2.5; NaCl 110.4; $CaCl_2$ 1.8; HEPES 5.0; pH 7.3. Contractures were elicited by application of Ringer's solution containing increased KCl and correspondingly reduced NaCl content. Chloride salts of divalent cations were added as described in text. The temperature of the bath solutions was kept at about 18°C by Peltier elements attached to the bottom of the muscle chamber. Perfusion solutions were adjusted accordingly.

Results

K Contractures at Normal Ca^{2+} Concentration

K contractures were similar in amplitude and time course to those recorded from slow fibers of the same species (Lüttgau, 1963; Nasledov et al., 1966) and *Xenopus laevis* (Lännergren, 1967). The contractile threshold was reached at a K concentration between 10 and 15 mM, and maximum tension (25 to 50 N/cm²) was obtained with 30 mM K (*cf.* Nasledov et al., 1966; Lännergren, 1967). At this K concentration tension was well maintained for at least 2 min. When 95 mM K-Ringer's was used, tension rose to the same peak values, but started to decline after about 20 sec (Fig. 1*b*). The amount of relaxation during K application varied considerably from fiber to fiber, but was reproducible within narrow limits when contractures were repeatedly evoked in the same fiber. In a series of 30 slow fibers the tension elicited by 95 mM K-Ringer's (and normal Ca^{2+} concentration) decreased from its peak value of 35.4 ± 8.2 N/cm² to 59.4 ± 23.9% (range 35 to 95%) after 1 min, and to 48.3 ± 27% (range 0 to 87%) by the end of the second minute (temperature 17 to 20°C). Thus, individual slow fibers differed markedly as to their ability to maintain tension.

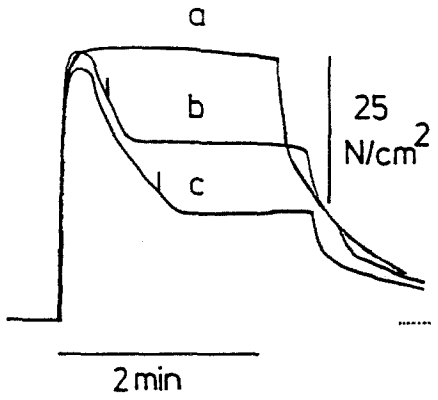

Fig. 2. Effect of Ca^{2+} added to external solution during K contracture. The fiber was equilibrated in normal Ringer's (1.8 mM Ca^{2+}). The Ca^{2+} concentration was increased to 10 mM (short vertical lines) at various times after beginning of contractures elicited with 95 mM K-Ringer's. Time intervals were 0 sec (*a*), 30 sec (*b*) and 60 sec (*c*), respectively. Intervals between records, 16 min; temperature, 18°C. Records superimposed photographically. Diameter of fiber, 68 μm

Influence of Ca^{2+} on the Time Course of K Contractures

When slow fibers were equilibrated for 2 min in Ringer's containing reduced Ca^{2+} concentrations, it was repeatedly observed that at concentrations below 0.2 mM resting tension increased. K contractures were therefore not examined in solutions containing less than 0.2 mM Ca^{2+}.

Figure 1 shows that peak tension decreased only slightly when the Ca^{2+} concentration was reduced from 1.8 to 0.2 mM (compare records *a* and *b*); relaxation was, however, much more pronounced in the low Ca^{2+} solution and resulted in an almost complete loss of tension by the end of the second minute. Similar results were obtained in another four slow fibers examined. When the contracture was performed at 10 mM Ca^{2+} (record *c*) the plateau value was reached somewhat later than at 1.8 mM Ca^{2+}, but tension decreased only slightly during the following 1 or 2 min. Altogether, the be-

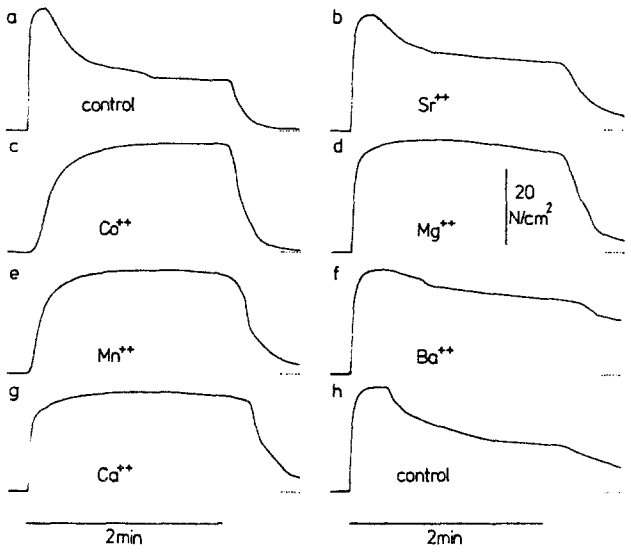

Fig. 3. Effect of various divalent cations on time course of K contracture. A slow fiber was equilibrated for 2 min in Ringer's containing 1.8 mM Ca^{2+} plus 10 mM of the divalent cations indicated; contractures elicited with 95 mM K-Ringer's containing the same concentrations of divalent cations as Ringer's used for equilibration; records *a* and *h* are control contractures in 95 mM K containing only 1.8 mM Ca^{2+}. Additional control contractures were performed between the test contractures in order to ascertain, that the effect of the divalent cation examined had vanished. Intervals between test contractures, 30 to 40 min; temperature, 18°C; all records from one slow fiber (diameter 75 μm). Note that all divalent cations improved maintenance of contracture tension

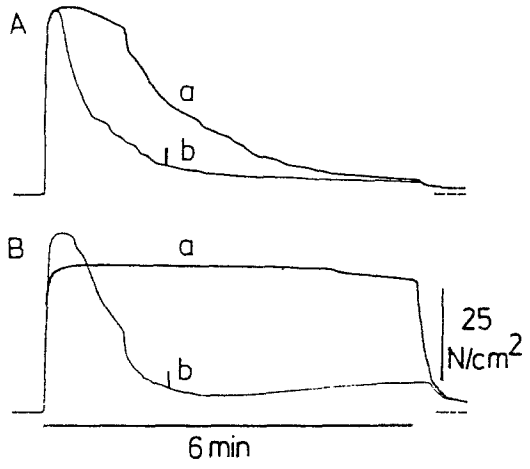

Fig. 4. Effect of 10 mM Mg^{2+} (*A*) or Ni^{2+} (*B*) on time course of K contracture. Records *a*, fiber equilibrated for 2 min in Ringer's containing 1.8 mM Ca^{2+} plus 10 mM Mg^{2+} (*A*) or Ni^{2+} (*B*). Contractures elicited with 95 mM K-Ringer's containing the same concentrations of divalent cations. Records *b*, fiber equilibrated in Ringer's containing 1.8 mM Ca^{2+}; 2 min after application of 95 mM K-Ringer's 10 mM Mg^{2+} or Ni^{2+} were added (vertical bars). Same fiber as in Fig. 2. Total duration of contractures, 6 min. temperature, 18°C. Note that addition of Ni^{2+} during K contracture only slightly increased tension (record *B*, *b*)

havior of *Rana temporaria* slow fibers closely resembled that described in detail for slow fibers of *Xenopus laevis* (Lännergren, 1967).

Effect of Ca^{2+} Added During K Contracture

An important question was whether Ca^{2+} would be able to restore contracture tension which had decreased in the course of prolonged K application. In Fig. 2 a slow fiber was equilibrated for 10 min in Ringer's containing normal Ca^{2+} (1.8 mM); then 95 mM K Ringer's was applied, and the Ca^{2+} concentration was increased to 10 mM after various intervals of time. In record *a* the interval was 0 sec, i.e. Ca^{2+} was added together with the high K concentration. Comparison with record *c* in Fig. 1 shows that the stabilizing effect of Ca^{2+} was rather fast, because the contracture tension hardly decreased during the following 2 min. When the Ca^{2+} concentration was raised 30 or 60 sec after the beginning of the contracture (records *b* and *c*), there was the usual initial rapid loss of tension, but further relaxation was stopped with a delay of 10 to 15 sec. Interestingly, the contracture was stabilized near the value which it had reached, but it was not restored to any appreciable extent by addition of Ca^{2+}.

Effect of Foreign Divalent Cations on Maintained Tension

Results similar to those illustrated in Fig. 1*c* were obtained when foreign divalent cations were added to the medium. Records obtained from two slow fibers are shown in Figs. 3 and 4. In both cases the fibers were equilibrated for 2 min in Ringer's containing 1.8 mM Ca^{2+} and 10 mM of the divalent cations indicated; the contracture solution contained the same concentrations of divalent cations. It is evident from these records, that all divalent cations improved maintenance of tension. Their effect was, however, quantitatively different, and it also varied from fiber to fiber, as is illustrated for the effect of 10 mM Mg^{2+} in Figs. 3 and 4. Two min after K application the tension was still 90% in Fig. 3*d*, but only 50% in the slow fiber of Fig. 4*A*, *a*. Similarly varying results were obtained with addition of Sr^{2+}, while the effects of Ni^{2+}, Co^{2+}, Mn^{2+} and Cd^{2+} did not vary to such an extent. These results are summarized in the Table. It can be seen that the most effective divalent cations were Ni^{2+}, Co^{2+} and Cd^{2+}; Mn^{2+} and Ca^{2+} ions were slightly less effective, and the least effective divalent cations were Mg^{2+}, Sr^{2+} and Ba^{2+}. Ni^{2+}, Co^{2+}, Mn^{2+} and Cd^{2+} in

Table. Effect of divalent cations on maintenance of contracture elicited by 95 mM K-Ringer's. Amplitude of contractures (in % of peak value) 2 min after application of contracture solution

Cation	Number of experiments	Amplitude of contracture (%)	
		A[a]	B[b]
Co^{2+}	6	25.0 ± 18.0	97.9 ± 3.3
Ni^{2+}	4	26.1 ± 16.7	97.4 ± 4.5
Cd^{2+}	3	62.8 ± 6.7	96.0 ± 3.7
Mn^{2+}	4	35.8 ± 14.6	92.3 ± 5.1
Ca^{2+}	6	33.3 ± 12.7	89.5 ± 9.2
Mg^{2+}	4	33.1 ± 20.7	73.1 ± 20.1
Sr^{2+}	5	35.0 ± 13.2	71.5 ± 13.7
Ba^{2+}	2	47.3	68.3

[a] Control contractures recorded in solution containing 1.8 mM Ca^{2+}.

[b] Contractures recorded in solution containing 1.8 mM Ca^{2+} and 10 mM divalent cations; all fibers equilibrated for 2 min in Ringer's containing the same concentrations of divalent cations.

addition to their stabilizing effect also slowed the rising phase of the contractures appreciably (for Co^{2+} and Mn^{2+}, *cf.* Fig. 3*c* and *e*). In two fibers the effect of Ni^{2+} on the K contracture was examined in the absence of Ca^{2+} (2-min preincubation in Ca-free Ringer's containing 10 mM Ni^{2+}), but amplitude and time course of the contracture were unchanged. Thus, the effect of foreign divalent cations does not depend on the presence of Ca^{2+} in the medium.

Addition of Foreign Cations during the K Contracture

Figure 4 shows an experiment, in which Mg^{2+} or Ni^{2+} was added to the contracture solution when tension had already dropped to a low value by 2 min after application of 95 mM K^+. After addition of Ni^{2+} there was a small and slow increase of tension (record *B, b*), which remained, however, far below the level maintained after preincubation (record *B, a*). In the same fiber addition of Mg^{2+} had no restoring effect (record *A, b*) and by the end of the 6-min contracture period tension was the same as after preincubation (record *A, a*). In several slow fibers these effects of divalent cations were compared. Addition of 10 mM Ni^{2+} (3 fibers) or Ca^{2+} (5 fibers) during the contracture restored tension by only 5% of the peak value, while upon application of 10 mM Co^{2+} (2 fibers) tension increased by 25%. In any case, tension was never restored to, or near the level which was maintained for 6 min after the fibers had been preincubated with the same cations (77 to 88% of peak). No restoring effect was observed when Mg^{2+} (3 fibers), Mn^{2+} (2 fibers) or Sr^{2+} (2 fibers) was added to the medium during the contracture.

Discussion

Two main results were obtained in the present investigation on K contractures of single slow fibers: Firstly, the maintenance of tension during prolonged depolarization by K^+ depends on external Ca^{2+}; secondly, foreign divalent cations have an effect qualitatively similar to that of Ca^{2+}. In *Rana temporaria* the effect of external Ca^{2+} on maintenance of contracture tension has been studied in muscles and bundles of muscle fibers (Pauschinger & Brecht, 1961; Schaechtelin, 1961) as well as in single slow fibers (Lüttgau, 1963; Nasledov et al., 1966; Gilly & Hui, 1980). The results obtained by these different groups of authors were equivocal (*see* Introduction). In the present experiments on 30 slow fibers of *Rana temporaria* external Ca^{2+} clearly improved maintenance of tension during prolonged K application. There were, however, large differences between individual fibers. This observation together with important methodological differences (exposure time in solutions with altered Ca^{2+} concentrations, duration of K contracture, addition of Mg^{2+} to low Ca^{2+} solutions) may explain at least part of the discrepancies between the results described in the literature.

The pronounced effect of external Ca^{2+} on maintenance of tension observed in the present experiments at first sight seems to support the view that there is a continuous influx of Ca^{2+} into the fibers during exposure to high K solutions. Such a mechanism is likely to operate in slow fibers of the chicken, whose ability to maintain tension is strongly impaired by removing external Ca^{2+}, or by adding Mn^{2+}, Co^{2+} or D600 to the medium (Page, 1969; Huerta & Stefani, 1981; Kikuchi & Schmidt, 1983). Similarly, in slow fibers of *Rana pipiens* the sustained phase of K contracture is reduced after removal of external Ca^{2+} or upon application of nifedipine (Huerta et al., 1986). Nifedipine has been shown to reduce Ca^{2+} currents (Almers, Fink & Palade, 1981), and slow fibers of *Rana pipiens* do have Ca^{2+} channels (Huerta & Stefani, 1986). These authors discuss the possibility that maintenance of tension is supported by an influx of Ca^{2+}; alternatively, binding of Ca^{2+} to superficial sites is regarded to be important. The latter mechanism seems to be essential in the slow fibers of *Rana temporaria,* because maintenance of tension is not only improved by external Ca^{2+}, but also by addition of foreign divalent cations (present results). The most effective divalent cations in this respect

are Co^{2+} and Ni^{2+}, which have been shown to block Ca^{2+} channels in twitch muscle fibers (Almers & Palade, 1981; Palade & Almers, 1985). In contrast, Mg^{2+} has a much smaller effect on Ca^{2+} channels, and its effect on maintained tension is also much less than that of Co^{2+} and Ni^{2+}. Thus, it may be concluded that contracture tension is best maintained under conditions of strongly reduced Ca^{2+} influx.

A similar conclusion was reached recently by Neuhaus (1987) in voltage-clamp experiments on frog twitch fibers; he blocked Ca^{2+} influx by application of nifedipine and found that the plateau phase of contractures was simultaneously prolonged.

On the other hand the order of efficiency of divalent cations on maintenance of contracture tension (Table) is almost identical with that described for their effect on surface charges in nerve fibers (Blaustein & Goldman, 1968; Hille, Woodhull & Shapiro, 1975; Brismar, 1980). Zacharova et al. (1985) found Ca^{2+} currents of small and widely varying amplitude in slow fibers of *Rana temporaria*; nonetheless, it must be concluded from the present results that maintenance of tension in slow fibers of this species does not depend on an influx of Ca^{2+} (*cf.* Miledi et al., 1981); in contrast, binding of Ca^{2+} or other divalent cations to superficial sites seems to be an important factor. Differences in binding affinity of the various cations (Hille et al., 1975) may be responsible for the observed differences in their effectiveness on maintained tension. In line with these conclusions are the observations on charge movement and Ca^{2+} release which have recently been reported by Brum et al. (1987). These authors conclude from their results that "a binding site, accessible from outside the cell, must be occupied for charge movement and Ca release."

The difference between the slow fibers of *Rana temporaria* and those of *Rana pipiens* is stressed by another observation. In the latter, addition of Ca^{2+} to the medium restores a considerable amount of the tension that had been lost in the course of a prolonged contracture, and Ni^{2+} cannot replace Ca^{2+} (Huerta et al., 1986). In slow fibers of *Rana temporaria*, only a small amount of lost tension can be restored, and this effect is exerted by Ca^{2+} as well as the Ca^{2+} channel blockers Ni^{2+} and Co^{2+}. Restored tension remains, however, far below the level observed after preincubation of the fibers with these divalent cations. Obviously, in *Rana temporaria*, but not in *Rana pipiens* slow fibers, the amount of tension loss during the K contracture seems to be determined predominantly by the concentration and species of divalent cations present at the time of activation of the contractile system. At least two possible mechanisms come into mind: loss or consumption of an activator or coupler substance (Hodgkin & Horowicz, 1960; Miledi, Parker & Zhu, 1983) could be controlled from an external site binding divalent cations; alternatively removal of Ca^{2+} from the myoplasma may be controlled similarly. The present results do not allow to distinguish between these possibilities, and more experiments are needed to elucidate the mechanisms underlying maintenance of tension in frog slow muscle fibers.

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References

Almers, W., Fink, R., Palade, P.T. 1981. Calcium depletion in frog muscle tubules: The decline of calcium current under maintained depolarization. *J. Physiol. (London)* **312:**177–207

Almers, W., Palade, P.T. 1981. Slow calcium and potassium currents across frog muscle membrane: Measurements with a Vaseline-gap technique. *J. Physiol. (London)* **312:**159–176

Blaustein, M.P., Goldman, D.E. 1968. The action of certain polyvalent cations on the voltage-clamped lobster axon. *J. Gen. Physiol.* **51:**279–291

Brismar, T. 1980. The effect of divalent and trivalent cations on the sodium permeability of myelinated nerve fibres of *Xenopus laevis*. *Acta Physiol. Scand.* **108:**23–29

Brum, F., Fitts, R., Pizarro, G., Rios, E. 1987. A Ca-Mg-Na site must be occupied for intramembrane charge movement and Ca release in frog skeletal muscle. *Biophys. J.* **51:**552a

Elul, R., Miledi, R., Stefani, E. 1970. Neural control of contracture in slow muscle fibres of the frog. *Acta Physiol. Latino Am.* **20:**194–226

Gilly, W.F., Hui, C.S. 1980. Mechanical activation in slow and twitch skeletal muscle fibres of the frog. *J. Physiol. (London)* **301:**137–156

Hille, B., Woodhull, A.M., Shapiro, B.I. 1975. Negative surface charge near sodium channels of nerve: Divalent ions, monovalent ions, and pH. *Philos. Trans. R. Soc. London B* **270:**301–318

Hodgkin, A.L., Horowicz, P. 1960. Potassium contractures in single muscle fibres. *J. Physiol. (London)* **153:**386–403

Huerta, M., Muñiz, J., Stefani, E. 1986. Effects of external calcium on potassium contractures in tonic muscle fibres of the frog (*Rana pipiens*). *J. Physiol. (London)* **376:**219–230

Huerta, M., Stefani, E. 1981. Potassium and caffeine contractures in fast and slow muscles of the chicken. *J. Physiol. (London)* **318:**181–189

Huerta, M., Stefani, E. 1986. Calcium action potentials and calcium currents in tonic muscle fibres of the frog (*Rana pipiens*). *J. Physiol. (London)* **372:**293–301

Kikuchi, T., Schmidt, H. 1983. Changes in resting and contractile properties of chicken muscles following denervation. *Biomed. Res.* **4:**303–314

Kuffler, S.W., Vaughan Williams, E.M. 1953. Properties of the 'slow' skeletal muscle fibres of the frog. *J. Physiol. (London)* **121:**318–340

Lännergren, J. 1967. The effect of calcium on potassium contractures of single slow muscle fibres of *Xenopus laevis*. *Acta Physiol. Scand.* **70**:16–25

Lehmann, N., Schmidt, H. 1979. Contractile responses to direct stimulation of frog slow muscle fibres before and after denervation. *Pfluegers Arch.* **382**:43–50

Lüttgau, H.C. 1963. The action of calcium ions on potassium contractures of single muscle fibres. *J. Physiol.* (*London*) **168**:679–697

Miledi, R., Parker, I., Schalow, G. 1977. Calcium transients in frog slow muscle fibres. *Nature* (*London*) **268**:750–752

Miledi, R., Parker, I., Schalow, G. 1981. Calcium transients in normal and denervated slow muscle fibres of the frog. *J. Physiol.* (*London*) **318**:191–206

Miledi, R., Parker, I., Zhu, P.H. 1983. Calcium transients studied under voltage-clamp control in frog twitch muscle fibres. *J. Physiol.* (*London*) **340**:649–680

Nasledov, G.A., Zachar, J., Zacharova, D. 1966. The ionic requirements for the development of contracture in isolated slow muscle fibres of the frog. *Physiol. Bohemoslov.* **15**:293–306

Neuhaus, R. 1987. The effect of nifedipine on slow Ca^{2+} inward current and force development in skeletal muscle fibres of the frog. *J. Physiol.* (*London*) **382**:122P

Page, S.G. 1969. Structure and some contractile properties of fast and slow muscles of the chicken. *J. Physiol.* (*London*) **205**:131–145

Palade, P.T., Almers, W. 1985. Slow calcium and potassium currents in frog skeletal muscle: Their relationship and pharmacologic properties. *Pfluegers Arch.* **405**:91–101

Pauschinger, P., Brecht, K. 1961. Influence of calcium on the potassium contracture of 'slow' and 'fast' skeletal muscle fibres of the frog. *Nature* (*London*) **189**:583–584

Schaechtelin, G. 1961. Der Einfluss von Calcium und Natrium auf die Kontraktur des M. rectus abdominis. *Pfluegers Arch.* **273**:164–181

Schmidt, H. 1987. Effect of divalent cations on the K-contracture of frog slow muscle fibres. *Pfluegers Arch.* **408**:Suppl. R82

Zacharova, D., Henček, M., Radzukiewicz, T.L., Zachar, J., Nasledov, G.A. 1985. Calcium currents recorded from segments of normal and denervated frog tonic muscle fibres. *Gen. Physiol. Biophys.* **4**:641–646

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