

Electromechanical Coupling II. The Effect of Perchlorate Upon Excitation-Contraction Coupling in Frog Skeletal Muscle Fibres

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In single skeletal muscle fibres perchlorate causes a large shift of the potential dependence of contraction activation to more negative potentials without a corresponding alteration in the kinetics of the inactivation process.

In two remarkable publications Foulks, Miller and Perry (1973) and Foulks and Perry (1979) investigated the effect of perchlorate upon excitation-contraction coupling. In the presence of this anion muscle fibres which had been transferred into a mechanically refractory state by full depolarization developed force when they were repolarized to -50 to -70 mV ("Repolarization-induced reactivation of contracture force"). From these and further findings the authors suggested that "tension appears to develop during repolarization when the reversal of inactivation occurs before the reversal of activation is completed".

In recent experiments with muscle fibres of the frog (*Rana temporaria*) we have confirmed and extended these results. We employed two methods: In a first series of experiments we used isolated single muscle fibres from the M. semitendinosus and induced twitches and contractures. The membrane potential was changed by altering the external potassium concentration at a constant KXCl product ($18-22$ °C; 1. method). In a second series we used single fibres in small bundles dissected from the M. lumbricalis digiti IV of the hind limb and employed a 2 microelectrode voltage clamp technique ($6-8$ °C; 2. method).

Results

1. The first sign of an increase in twitch height was observed at perchlorate concentrations around 10^{-4} M and the maximum effect upon twitches was reached near 10^{-1} M. The effect was reversible up to

concentrations of 10^{-2} M if perchlorate was applied for only a short period (1. method).

2. After the application of 8 mM-perchlorate muscle fibres, which had been passed through a cycle of force-activation and -inactivation ("spontaneous relaxation") induced by a sudden depolarization quickly regained up to 80% of their maximum force after a repolarization to -50 mV (1. method).

3. Perchlorate caused a parallel shift to more negative potentials of the S-shaped activation curve which relates peak force to the membrane potential. This shift reached about 30 mV in solutions containing 7 mM and 40 mV in those containing 70 mM perchlorate (2. method).

4. Preliminary experiments suggest that the potential dependence of the "steady state" inactivation curve and the rate of restoration of contractile activation remained unaltered after the application of 6–8 mM perchlorate (2. method).

5. From a selection of six further substances which resembled perchlorate in some physio-chemical properties only dichromate produced repolarization-induced contractures like perchlorate (1. method).

The results confirm the explanation of repolarization-induced contraction activation proposed by Foulks and Perry (1979). In addition they suggest that (a) spontaneous relaxation upon full depolarization is not due to an exhaustion of Ca-stores or of available energy reserves and that (b) the processes of contraction activation and inactivation are fairly independent of each other.

Perchlorate is of interest as a suitable tool for the analysis of the kinetics of EC coupling and the role of intramembrane charge movements for contraction activation because it reveals little or no effect upon the potential dependence of contraction *inactivation* and the contractile process itself. In addition, the

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steep potential dependence of the activation process is maintained even at high perchlorate concentrations. The latter is, for example, not the case with

caffeine which in high concentrations activates contraction more or less independently of the membrane potential.

[1] J. G. Foulks, A. D. Miller, and Florence A. Perry, Repolarization-induced reactivation of contracture tension in frog skeletal muscle. *Can. J. Pharmacol.* **51**, 324–334 (1973).

[2] J. G. Foulks and Florence A. Perry, The effects of temperature, local anaesthetics, pH, divalent cations, and group-specific reagents on repriming and repolarization-induced contractures in frog skeletal muscle. *Can. J. Physiol. Pharmacol.* **57**, 619–630 (1979).