HOW PERCHLORATE IMPROVES EXCITATION-CONTRACTION COUPLING IN SKELETAL MUSCLE FIBERS

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ABSTRACT The effect of the "chaotropic" anion, perchlorate, on the activation of contraction has been studied in voltage clamped frog skeletal muscle fibers. It was found that the voltage dependence of either the contractile force or the intramembrane charge movement was shifted towards more negative membrane potentials. The maximum values of force or charge movement attained with large depolarizing pulses did not change significantly. It is concluded that a specific perchlorate effect on the movement of charged particles can explain the potentiating effect of perchlorate anions on contractile force, strengthening the view that these charged particles serve as voltage sensors regulating Ca^{2+} release from the sarcoplasmic reticulum.

INTRODUCTION

Ten years ago Schneider and Chandler suggested that the contraction of skeletal muscle is controlled by charge movement within the membrane of the transverse tubular system (Schneider and Chandler, 1973). Here we report evidence in support of this concept. The large anion perchlorate facilitates the movement of charged particles during depolarizing voltage steps and increases the steepness of the curve that relates steady-state charge distribution between two positions in the membrane of transverse tubules. In the presence of 8 mM ClO_4^-, the midpoint voltage, $\overline{V}$, is shifted by 25 mV towards more negative potentials. This result may explain why perchlorate causes a corresponding shift of the potential dependence of force activation and an improvement in excitation-contraction coupling (Foulks et al., 1973; Fuxreiter et al., 1983).

Skeletal muscle fibers of the frog were used in all our experiments. Bundles of 5–10 fibers were dissected from the *m. lumbricalis digiti IV* of the frog's hind limb (*Rana temporaria*) and used for force measurements. The force produced by voltage clamp depolarization of an individual muscle fiber was determined. The fibers in this toe muscle are so short (~1.5 mm) that point voltage clamp with two internal microelectrodes can be applied with little potential decrement along the fiber (Caputo and Fernandez de Bolanos, 1979). To further improve the uniformity of the membrane potential we replaced external chloride by an impermeant anion and blocked ionic conductances with tetraethyl ammonium and tehodotoxin (TTX).

The effect of perchlorate on the activation of force is shown in Fig. 1. The toe fiber under investigation was kept at a holding potential of −110 mV for at least 5 min before it was suddenly depolarized for 30 s to the membrane potential indicated on the abscissa. Under normal conditions without perchlorate the first sign of activity was observed at −55 mV and maximum force was approached at about −40 mV, in approximate agreement with earlier measurements (Caputo and Fernandez de Bolanos, 1979). The application of 8 mM ClO_4^- caused a roughly parallel shift of the activation curve towards more negative potentials by ~20 mV. In further experiments, to be published in detail elsewhere (Gomolla et al., 1983), we found that the dose-response relation of this potential shift is steep at low perchlorate concentrations (up to 10 mM), reaching a 40 mV shift at 70 mM ClO_4^-. Interestingly, perchlorate hardly affects the potential dependence of force inactivation (Foulks et al., 1973; Fuxreiter et al., 1983). This leads to the restoration of force in depolarized, mechanically refractory fibers upon repolarization to potentials more negative than −50 mV, as first described by Foulks et al. (1973).

Intramembrane charge movements were measured in single fibers from the *m. semitendinosus* of *Rana esculenta*. The fibers were cut and voltage clamped using a single

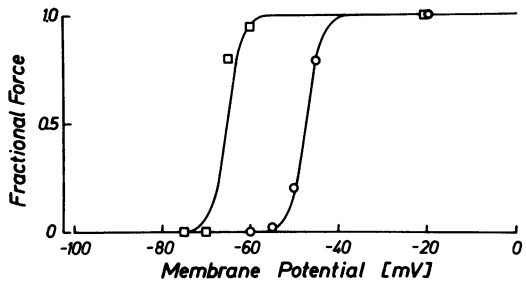

FIGURE 1 The effect of 8 mM ClO_4^- on the potential dependence of force activation under voltage clamp conditions. Holding potential −110 mV. Isometric force of the fiber under investigation was measured with a force transducer (Endevco Becton Dickinson & Co., San Juan Capistrana, CA, model 8107/2). The preparation was bathed in a solution containing 40.0 mM TEA sulphate, 1.25 mM K_2SO_4, 8 mM $CaSO_4$ (Ca^{2+} ~4 mM), 5 mM MOPS, 113 mM sucrose, and $5 \cdot 10^{-7}$ g/ml TTX (○), or a solution of the same composition except that 16 mM sucrose was replaced by 8 mM $NaClO_4$ (□). The temperature was 6°C.

gap technique (Kovács and Schneider, 1978). Ionic currents were suppressed with impermeant ions and channel blockers. The nonlinear capacity current responses to depolarizing pulses (I_Q) were obtained by subtracting both the ionic and linear capacity current components as described by Horowicz and Schneider (1981 *a*). Straight sloping baselines were fit to the last 50 points (1 ms/point) of the ON or OFF responses and subtracted from the entire ON or OFF to give the results presented here. The amount of charge displaced during each voltage step was obtained by integrating the current responses and expressed in relation to the linear capacitance of the fiber.

From a holding potential of −100 mV, depolarizing pulses to various test potentials were applied (Fig. 2).

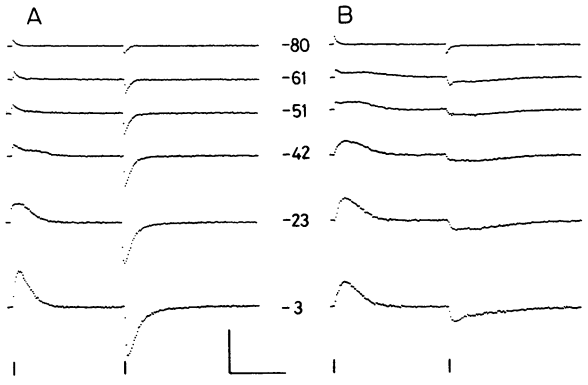

FIGURE 2 Records of nonlinear capacitive currents in cut semitendinosus muscle fibers. Depolarizing pulses of 100 ms duration (vertical bars) were applied from a holding potential of −100 mV to the test potentials indicated between traces in the control solution (*A*) and in the presence of 8 mM TEA-ClO_4 (*B*). The last 50 points of the OFF responses that were used for fitting the straight sloping baseline have been omitted from the figure. Time calibration represents 50 ms, vertical calibration corresponds to 2 μA/μF. External solutions (in mM): TEA^+ 150, Cs^+ 10, Ca^{2+} 8, SO_4^{2-} 88 and TTX in 10^{-7} g/ml. Internal solution (in mM): Cs^+ 120, Mg^{2+} 2, glutamate$^-$ 120, Cl^- 4, $EGTA^{2-}$ 1, ATP 0.5. The temperature was 2°–4° C.

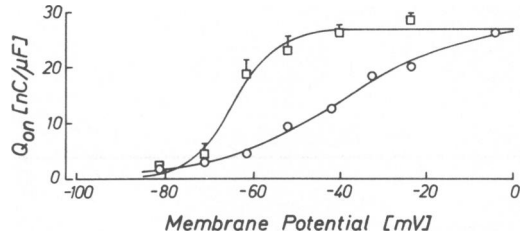

FIGURE 3 Effect of 8 mM ClO_4^- on charge movement during depolarizing pulses of 100 ms duration. Circles (○) and squares (□) give the mean values of Q_{ON} obtained from 5 fibers in the absence and presence of ClO_4^-, respectively. Error bars give SEM. The curves were drawn according to Eq. 1, with the parameter values given in Table I. The temperature was 2–4°C.

Without perchlorate, the first sign of an I_Q response became visible at depolarizing steps to about −80 mV, and the maximal response occurred beyond −40 mV. In the presence of perchlorate the maximum value was reached at more negative potentials. Although the overall time course of the ON response upon depolarization was significantly altered, the time constant of the declining phase was changed only slightly by perchlorate. In contrast, the time course of the OFF response upon repolarization was changed dramatically (Fig. 2). The OFF response exhibited a smaller peak and a marked prolongation of the declining phase. Nevertheless, the amount of charge transferred during the OFF response equaled (within 10% deviation) that of the corresponding ON response for all potentials, if care was taken to integrate the OFF response for a sufficiently long time (150 ms).

The Q_{ON} values averaged for five fibers were plotted against the membrane potential during the depolarizing steps in Fig. 3. If we postulate a Boltzmann distribution for a fixed amount of charge that can move freely between two positions inside the membrane (Schneider and Chandler, 1973), the amount of charge moved in response to a voltage step is given by the equation

$$Q = Q_{max}/[1 + \exp - (V - \bar{V})/k], \qquad (1)$$

where Q_{max} is the maximum charge, $\bar{V}$ the voltage at which 50% of the charge has moved and $1/k$ a steepness factor. Eq. 1 was fitted to the data using a nonlinear least-square procedure described by Scarborough (1966). The best fit, shown by the continuous lines in Fig. 3, was obtained for the parameter values in Table I. Our results show that the amount of movable charge, Q_{max}, is little if at all affected

TABLE I
PARAMETERS FOR FITTING VOLTAGE DISTRIBUTION OF CHARGE

	0 mM ClO_4^-	8 mM ClO_4^-
Q_{max} (nC/μF)	28.2	27.0
$\bar{V}$ (mV)	−39.5	−64.6
k (mV)	15.0	5.0

by perchlorate, but the charge moves at smaller depolarizations, leading to a steeper potential dependence of the charge movement. The main effect is a shift of $\bar{V}$ towards the resting potential by 25 mV.

Although the perchlorate effect is not entirely the same on the voltage dependence of charge movement and force activation, some considerations make the comparison possible. The overall voltage dependence of force activation is much steeper than that of the charge movement. The change from the contraction threshold up to the maximum activation occurs in a narrow, 10–15 mV potential range. The just visible contraction appears only if a certain amount of charge (~10 nC/μF) has moved (Horowicz and Schneider, 1981 *b*). After reaching the maximum level of contraction activation, the increase in depolarization can increase the amount of charge which moves without further increase of force, possibly due to the saturation of the contractile mechanism. Although we do not have enough data in this narrow potential range for direct comparison, the roughly similar shift (20–25 mV) observed in force activation and in the charge movement (in the midpoint voltage or at 10 nC/μF) makes it very probable that the perchlorate anions modify the E-C coupling of muscle fibers by influencing the charge movement process.

Perchlorate at concentrations below 10 mM appears to affect only the charge movement kinetics for the activation of force. It does not affect the potential dependence of delayed rectification, force inactivation or the threshold for the initiation of action potentials (Gomolla et al., 1983). Because the same effects were seen after internal application of perchlorate (by micro-injection or diffusion from the open-end pool), they cannot arise from adsorption to the outer membrane face which probably becomes significant only at much higher concentrations of the anion (McLaughlin et al., 1975). We therefore suggest that perchlorate enters the hydrophilic region of the transverse tubular membrane and in some way facilitates the movement of charged particles to their active position, while impeding their return to the resting state. The respective measuring conditions of the charge movement and force experiments may not be fully comparable. Yet the reasonable conformity of the results, together with the surprisingly specific and fully reversible action of perchlorate, supports the concept that the observed charges act as sensors within the transverse tubular membrane triggering the release of Ca^{2+} from the terminal cisternae of the sarcoplasmic reticulum.

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