
Observations on Intramembrane Charge Movements in Skeletal Muscle

W. Almers

Phil. Trans. R. Soc. Lond. B 1975 **270**, 507-513

doi: 10.1098/rstb.1975.0027

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Observations on intramembrane charge movements in skeletal muscle

BY W. ALMERS*

Physiological Laboratory, Cambridge CB2 3EG

Using signal-averaging techniques, one can record small membrane currents which remain even after blockage of the ionic currents which accompany electrical excitation in muscle. These residual currents probably represent the reorientation of charged molecules inside the membrane in response to a change in membrane potential. Two operationally separable types of intramembrane charge movement in muscle are described, one of which may play a role in excitation–contraction coupling. Studies of tetrodotoxin binding to muscle indicate that ‘sodium gating current’ is unlikely to contribute significantly to either type of charge movement.

When the large ionic conductances characteristic of the normal muscle fibre membrane are abolished, one can record small transmembrane currents which do not represent ion movements, but rather the movement of charged particles inside the membrane. Schneider & Chandler (1973) were the first to observe them, and pointed out that if these intramembrane charge movements occurred in the transverse tubular system, they could be important for excitation–contraction coupling by serving as a link of communication between tubules and sarcoplasmic reticulum. Although still unproven, this view is plausible because (i) both contractile activation and charge movement occur over a similar potential range and (ii) both disappear during maintained depolarization and reappear after hyperpolarization, and both do so with similar time courses (Chandler *et al.*, this volume). Dr R. H. Adrian & I have done some voltage-clamp experiments (Adrian, Chandler & Hodgkin 1970) on frog sartorius fibres in order to learn more about charge movements in muscle.

CHARGE MOVEMENTS AND EXCITATION–CONTRACTION COUPLING

We worked in the cold (4 °C) throughout and in a medium which contained 90 mM (tetraethylammonium)₂ sulphate, 5 mM Rb₂SO₄, 6.2 mM CaSO₄, 2 mM tris-maleate buffer of pH 7.1 and 0.2 μM tetrodotoxin; 350 mM sucrose was added to the solution to abolish contraction. The medium contains only Rb⁺ as a slightly permeable ion, and almost completely prevents time-dependent membrane conductance changes.

The typical experimental procedure was as follows. First, the currents during four small ‘control pulses’ were recorded, stored and summed in a PDP-8 computer. The pulses typically went from –108 to –92 mV and therefore did not include the range of potentials where the Schneider–Chandler currents are expected. Next, one ‘test pulse’ was given from the holding potential of –100 mV to some depolarized potential. This sequence was repeated 2–4 times for signal averaging. The computer then multiplied the summed current during the four control pulses by $B/4A$ and subtracted them point for point from the current during the test pulse; A is the amplitude of the control pulse, B that of the test pulse. What remained of the current

* Present address: Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195, U.S.A.

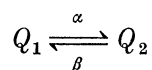
during the test pulse, the asymmetry current, was displayed on an oscilloscope, and its transient part taken to represent the intramembrane charge movement.

In agreement with Schneider & Chandler, we find that the asymmetry transients during and after the test pulse carry approximately equal charge. Typically, the ratio of on- and off-charges was unity with a standard error of 0.2. Plotting the average of on- and off-charges against the membrane potential during the test pulse gives sigmoid curves, which usually show saturation at positive potentials. In eight fibres, the steady-state dependence of charge displacement on the membrane potential showed the following characteristics; figures are given \pm standard error. (i) The 'maximal' charge displacement, as measured with test pulses to +30 mV, was 32.0 ± 7.9 nC/ μ F of membrane capacity. (ii) Charge displacement was half maximal at -45.8 ± 8.7 mV. (iii) Between -80 and -40 mV where charge displacement is relatively small, it increases e-fold in 12.2 ± 3.3 mV. The above results are in reasonable agreement with those obtained by others (Schneider & Chandler 1973; Chandler *et al.* 1975, this volume). They are consistent with the presence in the membrane of about 10^{11} charged particles per microfarad of membrane capacity, each with an effective valency of about two.

It has already been pointed out (Schneider & Chandler 1973) that the number of charged particles in a muscle fibre is very similar to that of close appositions between tubules and sarcoplasmic reticulum (Franzini-Armstrong 1970, who discovered them, called them 'feet'). If one accepts that the charge movement occurs there and serves as a trigger for Ca^{2+} -release, it is of interest how many Ca-ions the sarcoplasmic reticulum releases for every trigger molecule in the activating position. The myoplasmic concentration of sites which bind Ca^{2+} to troponin is about 0.1 mM (Ebashi & Endo 1968). Since it is extremely probable that within 40 ms from the beginning of a single action potential, the contractile mechanism of a frog sartorius at 0 °C is fully activated (Hill 1949), the sarcoplasmic reticulum must release at least 0.1 mM Ca^{2+} within less than 40 ms after arrival of the trigger particles in the activating position. In an average cylindrical fibre of 40 μ m radius and membrane capacity of 7 μ F/cm² of cylinder surface (see, for example, Adrian & Almers 1974) the membrane capacity per unit volume of muscle fibre is 3.5 F/l. With 10^{11} particles per microfarad, the concentration of trigger particles in muscle is about 0.5 μ M. If one assumes that Ca^{2+} release is one of the rate-limiting steps in contractile activation (Jöbsis & O'Connor 1966; Ebashi & Endo 1968; Ashley & Ridgway 1970) and also that on the average half of the trigger particles are in the activating position during the 40 ms latency period in Hill's experiment, it follows that the sarcoplasmic reticulum releases of the order of 400 calcium ions per activating particle in 40 ms, or about 10 000 ions per second and particle. For various reasons, this number may be too low, perhaps by as much as an order of magnitude. Nevertheless, a flux of 10^4 – 10^5 ions/s could easily be handled by a single ion-conducting 'pore'; in natural and artificial membranes, transit rates through pores of 10^6 – 10^8 ions/s are possible (Hladky & Haydon 1972; Gordon & Haydon 1972; Anderson & Stevens 1973; Hille 1970). However crude and speculative, the calculation shows that one would need only a single Ca^{2+} -permeable pore, perhaps in the immediate vicinity of each activating particle, in order to release sufficient Ca^{2+} for full and rapid activation of the contractile proteins after an action potential at 0 °C.

DO CHARGE MOVEMENTS FOLLOW FIRST ORDER KINETICS?

The simplest model for intramembrane charge movements will result if one assumes that a fixed number of charged particles (or dipoles) in the membrane can exist in two positions (or orientations) Q_1 and Q_2 ,



and that the movement between the two positions is determined by two rate constants α and β , which depend on the membrane potential only. Q_1 could be the resting, Q_2 the activating position. The mathematical analysis of this situation shows that after any perturbation of the system, the steady-state will be approached at a constant membrane potential V always via an exponential decline of charge movement with time constant $1/[\alpha(V) + \beta(V)]$.

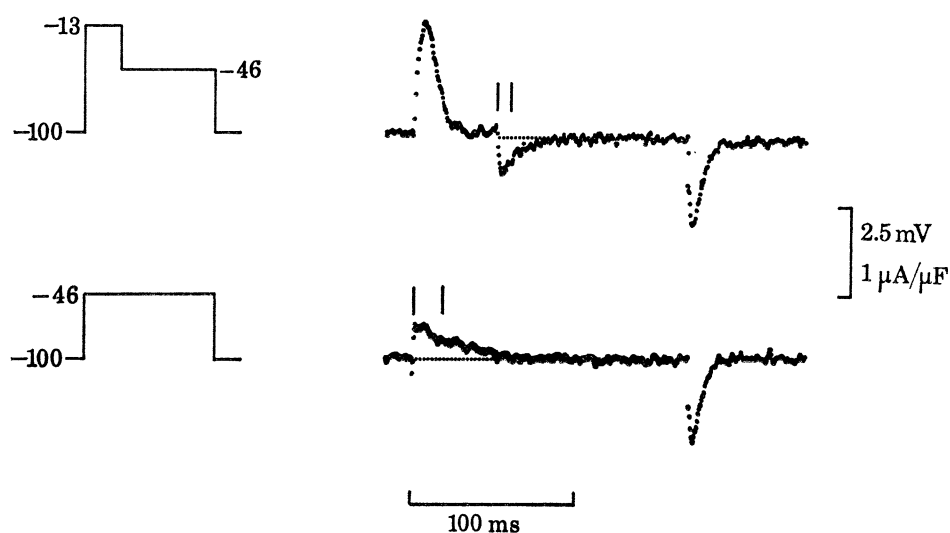


FIGURE 1. Asymmetry currents (right) during the test pulses drawn on the left; numbers are in mV. Control pulses were low-amplitude versions of the test pulses and went from -100 to -89 and -83 mV. Vertical lines above the current traces indicate the time at which half of the transient charge at -46 mV had flown; it is shorter in the upper (7.5 ms) than in the lower trace (17.5 ms). Charge displacements in $\text{nC}/\mu\text{F}$ are: upper trace, 18.2 outward at -13 mV, 6.8 return at -46 mV and 11.2 on return to -100 mV; lower trace 12.2 during and 10.5 after the pulse to -46 mV. Each trace was compiled from 20 control- and 4 test pulses.

Our observations cast some doubt on the applicability of this model to muscle. Especially at low Ca^{2+} -concentrations and at membrane potentials between -50 and -30 mV, asymmetry currents during a depolarizing test pulse often follow strikingly non-exponential time courses, and sometimes even rise to a maximum instead of declining monotonically. This maximum is sometimes visible on single oscilloscope current traces as a distinct 'hump' which is superimposed on the capacitive transient during the test pulse. The appearance on the single current trace rules out the possibility that the effect is caused in any way by the data-processing or signal averaging procedure.

Another observation which is difficult to explain by first-order kinetics, is shown in figure 1. In the upper trace most of the asymmetry charge was first transferred outward during a pre-pulse from -100 to -13 mV; some of the charge was then allowed to return inward at a potential of -46 mV. The lower trace shows asymmetry current during a single pulse from

–100 mV directly to –46 mV. It is clear that at –46 mV, outward movement of asymmetry charge is slower than its return after a pre-pulse.

Could such deviations from first order kinetics result from inadequate potential control in the transverse tubules? In the steady state, control in the tubules should be virtually perfect, because the membrane resistance in the solution used here is extremely high (Adrian & Almers (1974) obtained values of 20–30 k Ω cm² in a very similar solution). During the capacitive transient, however, one is not so fortunate. The variable of importance for the transient response in the tubules is the resistance in series with the tubule capacity. In the model of Adrian *et al.*

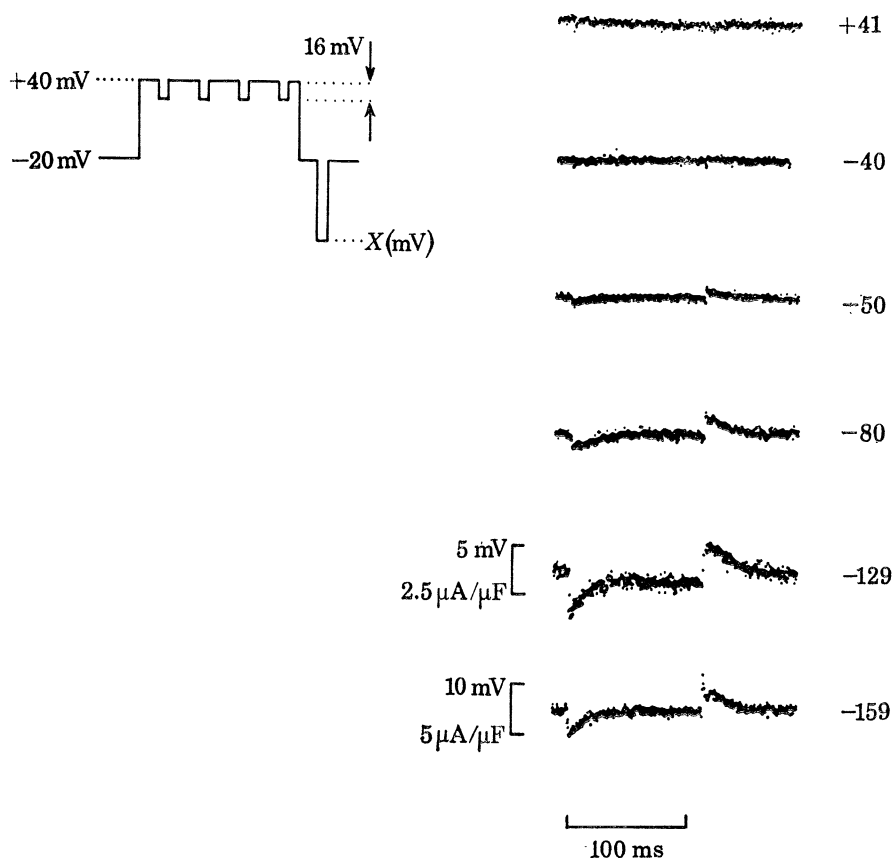


FIGURE 2. Asymmetry currents in depolarized fibres during test pulses from –20 mV to the potential indicated next to each trace. The sequence of 4 control- and 1 test-pulse is indicated on the left; it was applied twice for each trace. Holding potential –20 mV.

(1969) with the parameters proposed by Hodgkin & Nakajima (1972) for Ringer fluid, one would expect the peripheral three-quarters of the tubule membrane area in an 80 μ m diameter fibre to follow a step change in surface membrane potential to 90% within about 2 ms. This is too rapid to make a significant difference to the shape of the transients shown in figure 1. However, the conductivity of the medium used here is 5 mS/cm at 4 °C and about half that of Ringer fluid. Furthermore, the hypertonicity of the medium could have increased the tubule series resistance by as much as a factor of 2 or 3 (Almers, unpublished). Although still substantially less than the duration of asymmetry currents between –40 and –60 mV, the delays generated in the tubules may not be negligible in the present experiments. More analysis is needed to settle this point.

EVIDENCE FOR A SECOND TYPE OF INTRAMEMBRANE CHARGE MOVEMENT

Chandler and collaborators (Chandler *et al.* 1975, this volume) found that maintained depolarization abolishes asymmetry currents. We find the same, provided one uses control pulses in a potential range which is negative to the normal resting potential. If, however, one subtracts current during control pulses which go from say, +40 mV to +20 mV, then pulses to potentials more negative than about -50 mV produce clear asymmetry transients also in depolarized fibres (figure 2). Like the Schneider-Chandler transients, those in depolarized fibres show approximate equality of on- and off-charge, and we believe that they, too, represent intramembrane charge movements. As seen in figure 2, the rate at which this second type of charge moves does not depend very steeply on the membrane potential. The same is true for the steady-state distribution of the second component as a function of membrane potential (figure 3). This explains why the second charge does not appear if one takes control pulses negative to or around -100 mV. In that case, proportionally about the same asymmetry currents would flow during test and control pulses, and little or none of the second charge would remain after the subtraction.

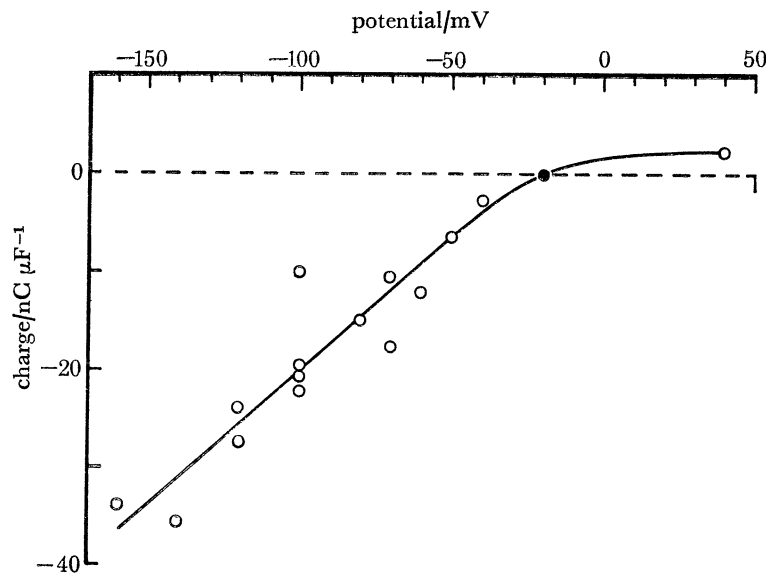


FIGURE 3. Steady-state relation between asymmetry charge and membrane potential in a depolarized fibre. Points are averages of on- and off-charges and are plotted positive when the transient during the test pulse was outward, negative when it was inward. Same experiment as in figure 2.

In four experiments of the type described, the amount \pm standard error of charge displaced at -150 mV was 26.3 ± 5.5 nC/ μF , which is similar to the maximal amount of asymmetry charge in polarized fibres. In spite of the size of this second component, its physiological significance is still unclear. Its time- and voltage-dependence make it difficult to see how it could have anything to do with, for instance, the regulation of sodium- or potassium-permeability during the action potential.

CONCLUDING REMARKS

The results presented here can be summarized as follows:

(i) If the Schneider–Chandler currents represent the movement of a single population of charged particles or dipoles, then their motion most probably does not follow first order kinetics.

(ii) There is not one, but at least two operationally separable types of intramembrane charge movement in skeletal muscle.

The two findings clearly raise more questions than they answer. However, neither need surprise us. If the Schneider–Chandler charge does represent the physiological link of communication across the junction between tubules and sarcoplasmic reticulum, it is probable that one is dealing with a molecular interaction over substantial distances. The forces and restraints acting on a long ‘trigger molecule’ which extends some ways across the junction may well be rather more complicated than those which govern, e.g. the ‘gating’ process for sodium permeability in nerve. The latter takes place in a space less than 5 nm wide, whereas the electron-microscopic thickness of the triadic junction is some 20 nm (Franzini-Armstrong 1970). The discovery of a second charge movement with as yet no obvious physiological function is likewise not surprising, since any permanent molecular dipole inside the membrane – e.g. a protein molecule – could in principle give rise to such an effect.

One might ask why there is no indication in frog sartorius muscle of any charge movement which is comparable to that seen in squid nerve (Armstrong & Bezanilla 1973; Keynes & Rojas 1973) and none which could be related to the regulation of excitatory Na⁺ permeability. The answer to this question depends on the number of Na⁺ channels in frog sartorius muscle. Using a desheathed frog sciatic nerve as a bioassay preparation, Dr R. S. Levinson & I have recently measured tetrodotoxin binding to frog sartorius muscle. We found a distinct saturable population of tetrodotoxin binding sites with an association constant of 5 nM, which numbers around 300–400 sites/ μm^2 surface of a cylinder 80 μm in diameter. Since the dependence of sodium conductance on potential suggests that there are the equivalent of 4–6 movable charges per channel (Hodgkin & Huxley 1952; Frankenhaeuser 1960), one would expect a maximal sodium gating charge of some 3–5 nC/ μF in frog muscle. This is about 1/10 to 1/7 of the asymmetry charge observed, and could easily have gone unnoticed.

This work was done during the tenure of a research fellowship of Muscular Dystrophy Associations of America.

REFERENCES (Almers)

- Adrian, R. H. & Almers, W. 1974 Membrane capacity measurements on frog skeletal muscle in media of low ion content. *J. Physiol., Lond.* **237**, 573–605.
- Adrian, R. H., Chandler, W. K. & Hodgkin, A. L. 1969 The kinetics of mechanical activation in frog muscle. *J. Physiol., Lond.* **204**, 207–230.
- Adrian, R. H., Chandler, W. K. & Hodgkin, A. L. 1970 Voltage-clamp experiments in striated muscle fibres. *J. Physiol., Lond.* **208**, 607–644.
- Anderson, C. R. & Stevens, C. F. 1973 Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol., Lond.* **235**, 655–692.
- Armstrong, C. M. & Bezanilla, F. M. 1973 Currents related to movement of the gating particles of the sodium channels. *Nature, Lond.* **242**, 459–461.
- Ashley, C. C. & Ridgway, E. B. 1970 On the relationships between membrane potential, calcium transient and tension in single barnacle muscle fibres. *J. Physiol., Lond.* **209**, 105–130.

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- Ebashi, S. & Endo, M. 1968 Calcium ion and muscle contraction. *Progr. Biophys. molec. Biol.* **18**, 123–183.
- Frankenhaeuser, B. 1960 Quantitative description of sodium currents in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol., Lond.* **151**, 491–501.
- Franzini-Armstrong, C. 1970 Studies of the triad. I. Structure of the junction in frog twitch fibres. *J. Cell Biology* **47**, 488–499.
- Gordon, L. G. M. & Haydon, D. A. 1972 The unit conductance channel of alamethicin. *Biochim. biophys. Acta* **255**, 1014–1018.
- Hill, A. V. 1949 The abrupt transition from rest to activity in muscle. *Proc. R. Soc. Lond. B* **136**, 399–420.
- Hille, B. 1970 Ionic channels in nerve membranes. *Progr. Biophys. molec. Biol.* **21**, 1–32.
- Hladky, S. B. & Haydon, D. A. 1972 Ion transfer across lipid membranes in the presence of gramicidin A. I. Studies of the unit conductance channel. *Biochim. biophys. Acta* **274**, 294–312.
- Hodgkin, A. L. & Huxley, A. F. 1952 A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., Lond.* **117**, 500–544.
- Hodgkin, A. L. & Nakajima, S. 1972 Analysis of the membrane capacity in frog muscle. *J. Physiol., Lond.* **221**, 121–136.
- Jöbsis, F. F. & O'Connor, M. J. 1966 Calcium release and reabsorption in the sartorius muscle of the toad. *Biochem. biophys. Res. Commun.* **25**, 246–253.
- Keynes, R. D. & Rojas, E. 1973 Characteristics of the sodium gating currents in the squid giant axon. *J. Physiol., Lond.* **233**, 20P.
- Schneider, M. F. & Chandler, W. K. 1973 Voltage-dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature, Lond.* **242**, 244–246.