Impedance of Membrane and Myoplasm during the Action Potential of Frog Muscle

W. H. FREYGANG and R. GUNN

From the Division of Biological and Biochemical Research, National Institute of Mental Health, Bethesda, Maryland 20014

ABSTRACT The transient imbalance of a Wheatstone bridge was used to estimate the changes in both membrane and myoplasmic impedance that occurred as an action potential propagated over a 1 mm length of a frog muscle fiber. It was also used to estimate the changes in the membrane impedance alone. Alterations in myoplasmic impedance that might have been predicted were not found.

INTRODUCTION

The aim of these experiments was to relate changes in impedance of the membrane and the myoplasm to the action potential of frog muscle. Since it was possible to do these experiments on isolated muscle fibers, it was of interest to look for impedance changes in the myoplasm that might reflect on the mechanism of the linkage between the electrical and contractile activities of the cell.

METHODS

The experiments were done on either sartorius muscles or on single muscle fibers that had been isolated from the semitendinosus muscle of *Rana pipiens*. Impedance was measured from a balanced Wheatstone bridge. The fixed resistance arms of the bridge were in the ratio of either 10:1 or 100:1. Two methods of measurement were employed. One method was designed to be sensitive to the myoplasmic impedance and the other measured primarily the impedance of the plasma membrane. With both methods the signal applied to the Wheatstone bridge was usually 10 kHz. The output of the bridge was amplified and displayed on a cathode ray tube directly, or passed through a tuned filter (Model 330MR, Krohn-Hite Corp., Cambridge, Mass.) and then displayed. The variable arm of the bridge consisted of a variable resistance and a variable capacity connected in parallel. The bridge could detect a 0.1% change in the resistance of the arm that was connected to the preparation.

The magnitude of the imbalance of the bridge is proportional to the change in the impedance of the unknown arm when the bridge remains close to balance (Cole and Curtis, 1939).
The action current that flowed through the bridge produced only a very small ringing of the bridge that occurred at the time of the peak of the action potential. It could be seen when the 10 kHz signal was turned off.

**Fine Wire Method**

This method measured the impedance of the surface membrane. The recording situation is drawn in Fig. 1. A 25 μm platinum wire insulated with teflon was coated with platinum black at its tip. The tip of the wire was pressed against individual muscle fibers in the intact sartorius muscle. This wire and a reference wire in the surrounding solution were led to the Wheatstone bridge. The muscle fiber was stimulated by two platinum wires 0.2 mm apart that were insulated to their tips with Araldite (CIBA [A.R.L.], Ltd., Duxford, Cambridge, England). Stimuli were obtained from a stimulus isolation unit. A glass micropipette filled with 3 M KCl was used to impale the cell at the location of the impedance measurement. The signal from the micropipette was led to a Bak amplifier (Model M3, Cooke Engineering Co., Alexandria, Va.). Thus the action potential and the impedance changes could be recorded simultaneously.

We calculate that the space constant for a 10 kHz signal in an average muscle fiber is about 100 μm. Since the spread of the signal is of the order of a fiber diameter, it seems reasonable to consider the contribution of the myoplasm to the impedance as negligible.

**Gap Method**

Fig. 2 illustrates this method, which has been used by Cole and Hodgkin (1939) for measurements on squid axons and by Tamasige (1950), who found an air gap to be
better suited to single muscle fibers than the oil gap employed by Cole and Hodgkin. The preparation was suspended in the air gap between the bottom of the test tube and the solution in the lower container. The upper tendon was tied to a thread that, at first, ran through a hole in the bottom of the test tube. By pulling the thread, the end of the muscle fiber was pulled into the test tube. A platinum weight was tied to the lower tendon in order to maintain tension. The gap was made by raising the test tube out of the fluid in the lower container. A Plexiglas cylinder (Rohm and Haas Co., Philadelphia, Pa.) that surrounded the apparatus provided protection from air currents. The two platinum black wires, one in the test tube and the other in the lower container, were led to the Wheatstone bridge. Two insulated platinum wires were glued to the outside of the test tube. The uninsulated ends of these wires faced each other across the hole in the bottom of the test tube. They
were connected to a stimulus isolation unit and were used to excite the preparation. This method measures the impedance of both the plasma membrane at the ends of the gap and the impedance of the myoplasm within the gap. When the length of the preparation in the gap is long in comparison to the space constant for alternating current, the contribution of the membrane to the total impedance is small, especially at high frequencies. For example, the resistance of a millimeter length of a fiber having a diameter of 80 μm is about 400,000 Ω. The sum of the impedances of the membrane at the two ends of the gaps should be less than 20,000 Ω.

**Solutions**

The Ringer’s fluid had the following composition (millimoles/liter): K⁺, 2.5; Na⁺, 120; Ca²⁺, 1.8; Cl⁻, 121; HPO₄²⁻, 2.15; H₂PO₄⁻, 0.85. Either 232 or 350 mM sucrose was often added to the Ringer’s fluid to suppress contraction. The transverse tubular system was disrupted and contraction almost eliminated by immersing the preparation for an hour in a Ringer’s fluid to which 400 mM glycerol had been added and then returning it to Ringer’s fluid, as described by Howell (1969).

**RESULTS**

**Fine Wire Method**

Fig. 3 shows a simultaneous recording of the bridge imbalance and the action potential from a fiber in 232 mM sucrose Ringer’s fluid. The two records may be considered as arising from the same part of the muscle fiber since the fiber was impaled by the micropipette less than 10 μm away from the tip of the wire. The bridge imbalance consists of two components. The first component occurs during the rising phase, peak, and start of the falling phase of the action potential. The second component appears during the falling phase of

![Figure 3](image-url)  
*Figure 3. Simultaneous recordings of the bridge imbalance (upper trace) and the action potential (lower trace) from a sartorius muscle fiber. The fine wire method was employed. Sucrose (232 mM) was added to the Ringer’s fluid. 21°C.*
the action potential and lasts well into the early afterpotential. Between the two components the bridge approached its balanced state. The bridge could be balanced during either component by reducing the parallel resistance in its variable arm. Increasing the parallel resistance, or altering the variable parallel capacity in this arm in either direction, produced a constant amount of imbalance to which the transient imbalances were added. Thus both components in Fig. 2 appear to be the result of a decrease in the impedance of the plasma membrane. As we pointed out above, the myoplasmic impedance plays a negligible role in these measurements.

Treatment with glycerol produced records like those in Fig. 3. The absence of sucrose also produced the same shape of bridge imbalances except that the bridge went out of balance when the muscle twitched.

**Gap Method**

The impedance change during an action potential as registered by the gap method is illustrated in Fig. 4. This experiment was done at 14°C in order to slow the transient changes in the membrane impedance. Two transients in membrane impedance are shown in Fig. 4. The first occurred as the action potential passed the Ringer-air junction at the bottom of the test tube and the second appeared when the action potential reached the Ringer's fluid in the lower container. Each of these two transients are similar to the first imbalance in Fig. 3. The time between the two early imbalances increased with the length of the air gap. They are followed by a second imbalance like that

![Figure 4](image_url)

**Figure 4.** Bridge imbalance during an action potential as recorded from a single semitendinosus muscle fiber in an air gap. Lower trace is the output from the bridge and shows the action current with the signal from the bridge. This signal has been filtered and amplified to produce the upper trace. Gap was approximately 1 mm. Sucrose (232 mM) was added to the Ringer's fluid. 14°C.
shown in Fig. 3. The similarities in Figs. 3 and 4 lead us to regard both sets of records as indications of the decreases in impedance of the plasma membrane. Movement of the muscle fiber was eliminated in this experiment by the presence of sucrose in the Ringer's fluid. Under these conditions it appears that the myoplasm of the portion of the fiber in the air gap did not display a change in impedance. Records similar to those in Fig. 4 were obtained at room temperature, except that the two early imbalances were less separated in time.

When the muscle was allowed to twitch there was the large imbalance that occurred during the contraction, as can be seen at the ends of the records in Fig. 5. It is a fall in impedance caused by the muscle pulling itself up into the air gap. Otherwise the records were similar to those obtained when the muscle did not twitch in the air gap (Fig. 4) and with the fine wire method (Fig. 3). There was more variability in the time-course of the second imbalance when the muscle was allowed to twitch. This probably was caused by the geometrical changes associated with the latency relaxation and contraction. The magnitude of the latency relaxation is reduced in hypertonic solutions (Matsushita, 1969). Clearly no other type of impedance changes occurred when the muscle was allowed to twitch. The use of a longer length of muscle in the gap in order to exaggerate a component that might arise from the myoplasm was not successful in showing it. The result of using a longer length in the gap was to reduce the sensitivity because of the added series resistance of the longer length of myoplasm.
DISCUSSION

Membrane Impedance Change with the Action Potential

The two components of the bridge imbalance were both produced by a fall in the impedance of the unknown arm of the bridge. Adrian, Chandler, and Hodgkin (1970) have estimated the rise in the conductance of the membrane of the muscle fiber from voltage clamping experiments. It seems likely that the decreases in impedance measured in the experiments reported here are reflections of the same process. A frequency of 10 kHz was employed in our experiments and therefore only an insignificant amount of the alternating current could have flowed into the transverse tubular system (Falk and Fatt, 1964). Therefore the bridge imbalances that we observed should be produced primarily by changes in the conductance of the surface membrane.

The relation between our records of the imbalance of the bridge and the changes in membrane conductance is not simple. It will depend upon the relative magnitudes of the membrane impedance and the impedance of the shunt around the membrane while the membrane impedance changes during the action potential. By analogy with the experiments of Cole and Curtis (1939) on the giant axon of the squid, which are geometrically somewhat similar to the experiments we made, one might take our bridge imbalances to be proportional to changes in membrane conductance, but that could be a hazardous assumption. It is safer to consider the imbalances as simply a monotonic, but unknown, function of membrane conductance.

The data in Fig. 6 illustrate the percent change in membrane conductance estimated by Adrian et al. (1970). The action potential that was calculated for this conductance change is also drawn in the figure. The agreement between the results of the two types of experiments is fairly close, as can be seen by comparing Fig. 3 with Fig. 6. The notch that separates the first and second

![Figure 6](image-url)

Figure 6. Solid line is the action potential and the dotted line is the sum of $g_{Na}$ $g_{K} + g_{NaK}$ shown as a percent of its maximum value. For a propagated action potential at 20°C. Data from Adrian, Chandler, and Hodgkin (1970) and kindly supplied by Dr. Chandler.
imbalances is absent in the data that were derived from the voltage clamp. The reason for the discrepancy is not apparent to us. Sucrose-Ringer or the glycerol treatment did not alter the time-course and shape of the bridge imbalances that we recorded with the fine wire method. We conclude from this that the superficial membrane of the cells was not altered in this respect by these factors.

**Absence of Detectable Changes in Myoplasmic Impedance**

We cannot give an accurate estimate of the smallest change in myoplasmic impedance that we could have seen because of an unknown amount of fluid that adhered to the single fibers. As an approximation, however, we could detect a 1% change in impedance of the myoplasm, as a conservative estimate. The liberation of ionized calcium in the myoplasm that is necessary to initiate a contraction has been estimated to be $10^{-6}$ M (Hellam and Podolsky, 1969). The small increase in conductance associated with this rise in calcium concentration could not be detected by our bridge. The terminal cisternae, the intermediate cisternae, and the longitudinal tubules together with the fenestrated collar each have a volume of 4–5% of the fiber volume (Peachey, 1965) in frog's sartorius muscle. One might expect that a change in the conductivity of these structures that might be associated with the development of the active state would be reflected in the myoplasmic impedance. The observations here do not rule this possibility out. We know too little about the electrical properties of these intracellular structures to estimate how large the change in myoplasmic impedance might be. They do suggest that other sources for the postulated rise in myoplasmic calcium concentration should still be considered.

Received for publication 21 July 1972

**REFERENCES**

Adrian, R. H., W. K. Chandler, and A. L. Hodgkin. 1970. Voltage clamp experiments in striated muscle fibres. *J. Physiol. (Lond.)* 208:607.

Cole, K. S., and H. J. Curtis. 1939. Electric impedance of the squid giant axon during activity. *J. Gen. Physiol.* 22:649.

Cole, K. S., and A. L. Hodgkin. 1939. Membrane and protoplasm resistance in the squid giant axon. *J. Gen. Physiol.* 22:671.

Falk, G., and P. Fatt. 1964. Linear electrical properties of striated muscle fibres observed with intracellular electrodes. *Proc. R. Soc. Lond. B Biol. Sci.* 160:69.

Hellam, D. C., and R. J. Podolsky. 1969. Force measurements in skinned muscle fibres. *J. Physiol. (Lond.)* 200:807.

Howell, J. N. 1969. A lesion of the transverse tubules of skeletal muscle. *J. Physiol. (Lond.)* 201:515.

Matsumura, M. 1969. On the nature of the latency relaxation of frog skeletal muscle. *Jap. J. Physiol.* 19:701.

Peachey, L. D. 1965. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. *J. Cell Biol.* 25(3, Pt. 2):209.

Tamasige, M. 1950. Membrane and sarcoplasm resistance in an isolated frog muscle fibre. *Ann. Zool. Jap.* 23:125.