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Low membrane resistance in sucrose gap – a parallel leakage path

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SUMMARY

The high resistance lobster axon appears to be very leaky under voltage clamp in sucrose gap because of a parallel leakage current. The parallel current rectifies and depends on holding potential.

The giant axon from the circumesophageal connective in lobster (*Homarus americanus*) has a large resting membrane resistance. Brinley[1] obtained an average value of 8257 $\Omega \cdot cm^2$ using microelectrodes. In conductance units this is 0.121 $k\Omega^{-1} \cdot cm^{-2}$. In double sucrose gap[2,3] one would expect the steady-state leakage conductance for small voltage clamped step depolarizations about a hyperpolarized holding potential to be on average no larger than 0.121 $k\Omega^{-1} \cdot cm^{-2}$. Measured values, however, are more than two orders of magnitude greater than this. Narahashi *et al.*[4] found 22 $k\Omega^{-1} \cdot cm^{-2}$, Blaustein and Goldman[5] found 18 $k\Omega^{-1} \cdot cm^{-2}$, while analysis of our previously published data[6] for leakage yielded a figure of 19 $k\Omega^{-1} \cdot cm^{-2}$. These values are in good agreement but leave the question as to why leakage conductance in sucrose gap should be so high. It is evident that the sucrose gap either systematically and precipitously opens leakage channels in the membrane or it introduces a systematic error in the measurement of membrane current.

We have examined leakage current in lobster axons using the standard double sucrose gap voltage clamp technique[2,3]. Our data show that a given voltage clamped step change in membrane potential drives a leakage current which is independent of the membrane area in the gap between the sucrose streams. This means that the vast majority of the leakage current flows in parallel with, rather than through, the membrane area in the gap. This parallel current swamps the 'real' leakage current, which presumably still

varies with membrane area, and yields an equivalent conductance some 170 times the predicted leakage conductance. Thus the unexpectedly high leakage conductance in sucrose gap may be explained by the existence of an area-independent current flowing in parallel with the membrane in the gap.

Leakage current independence of membrane area is demonstrated in double pulse experiments which allow simultaneous observation of leakage and sodium currents. Starting from a holding potential of −100 mV, the membrane is repeatedly clamped to −80 mV for several milliseconds to observe outward leakage current, and then to 0 mV, to observe inward sodium current, while the area is changed. As the area is varied over wide limits the leakage currents at −80 mV do not change, while the inward sodium at 0 mV varies directly with the area, as expected. An example of this is shown in Fig. 1,

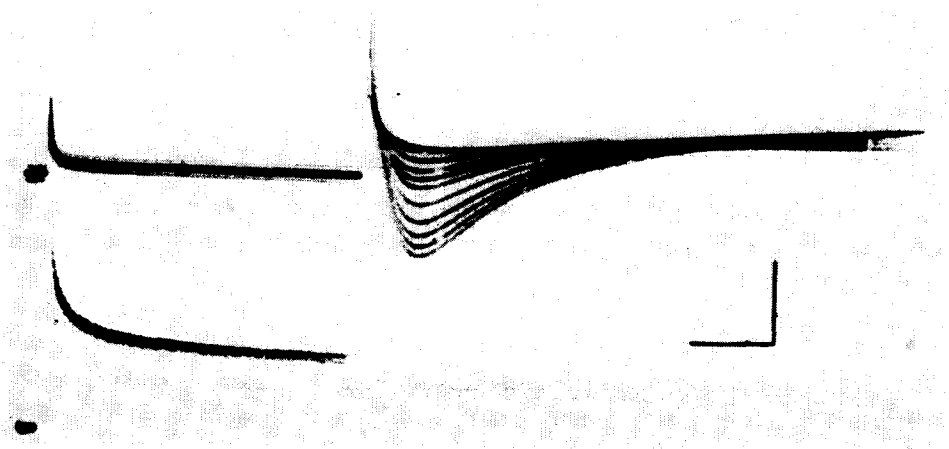

Fig. 1. Superimposed photographs of oscilloscope tracings of currents in voltage clamp associated with step depolarizations to −80 mV and then to 0 mV from a holding potential of −100 mV, taken as the sucrose gap area was varied. The lower trace is the same as the upper trace, but at 10× higher sensitivity to allow resolution of the currents at −80 mV. At 0 mV the lower trace was too fast to photograph and was mostly off scale. The calibration scale is 0.4 μA and 1 msec (upper trace) and 0.04 μA and 1 msec (lower trace).

which is a photograph of superimposed sweeps of current *vs* time on a dual beam oscilloscope. The lower beam is at 10 times the sensitivity of the upper beam so that the current at −80 mV can be seen clearly. When the area was set at its smallest value there was virtually no sodium current, yet the leakage remained as large as when the area was set at its greatest value. For the axon in Fig. 1, a 20-mV depolarization drove a final current of 0.04 μA. The assumption of a normal gap area for this 100-μm axon leads to a leakage conductance of 13 $m\Omega^{-1} \cdot cm^{-2}$, a figure well within the normal range. Other experiments with depolarizations to more positive potentials have shown that potassium current also varies with area.

The magnitude of the measured leakage current depends on the between pulse holding potential and it rectifies about the holding potential. As shown in Table I, the

TABLE I

RATIOS OF CURRENT (± S.E.) AT INDICATED HOLDING POTENTIAL TO THAT AT −100 mV, FOR 30-mV STEP POTENTIAL CHANGES, AT 0.5 msec AND 4 msec AFTER START OF POTENTIAL STEP

Results are from 12 axon areas

Holding potential (mV)	*Ratio for +30 mV step*		*Ratio for −30 mV step*	
	At 0.5 msec	*At 4 msec*	*At 0.5 msec*	*At 4 msec*
−110	0.96±0.006	0.91±0.008	0.98±0.006	0.94±0.005
−120	0.93±0.015	0.87±0.013	0.94±0.015	0.89±0.011
−130	0.91±0.019	0.81±0.015	0.93±0.015	0.85±0.015

current resulting from 30-mV step changes becomes smaller as the holding potential is made more negative. This is true for both positive and negative steps. A negative 30-mV step from −130 mV, for example, drives only 85% of the current after 4 msec as is driven by an equal step from −100 mV. The dependence of leakage conductance on the holding potential is less noticeable at 0.5 msec, but it is always seen. We also find that currents for a given step and holding potential are always greater for depolarizing steps, as shown in Table II. The rectification effect may be related to the normal delayed rectification

TABLE II

RATIOS OF OUTWARD TO INWARD CURRENT (± S.E.) FOR SYMMETRICAL 30-mV STEP POTENTIAL CHANGES ABOUT HOLDING POTENTIAL, AT 0.5 msec AND 4 msec AFTER START OF POTENTIAL STEP

RESULTS ARE FROM 12 AXON AREAS.

Holding potential (mV)	*Ratio at 0.5 msec*	*Ratio at 4 msec*
−100	1.08±0.01	1.22±0.017
−110	1.07±0.008	1.18±0.018
−120	1.06±0.005	1.18±0.019
−130	1.07±0.011	1.16±0.019

properties of the axon, but it is seen here at times as short as 0.5 msec and at potentials considerably hyperpolarized from the potentials at which one can discern a potassium current separate from leakage. Comparison of this rectification data with that obtained on other preparations not using sucrose gap[7,8] may not be meaningful because of the different origin of the currents.

Calculations from sucrose resistance and axon chamber dimensions show that a direct current path between chamber pools through the sucrose cannot account for the observed magnitude of leakage current. A potential-dependent leeching of ions into the sucrose from the axon may be responsible.

It seems likely that a parallel current should flow with squid axons in sucrose gap also. This is supported by the finding that squid leakage conductance in sucrose gap is considerably higher than squid leakage conductance with the internal wire method[9].

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