Leak Current Rectification in *Myxicola* Giant Axons

*Constant field and constant conductance components*

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**ABSTRACT** Early leak current, i.e. for times similar to the time to peak of the transient current was measured in *Myxicola* giant axons in the presence of tetrodotoxin. The leak current-voltage relation rectifies, showing more current for strong depolarizing pulses than expected from symmetry around the holding potential. A satisfactory practical approximation for most leak corrections is constant resting conductance. The leak current-voltage curve rectifies less than expected from the constant field equation. These curves cannot be reconstructed by summing the constant field currents for sodium and potassium using a $P_{Na}/P_{K}$ ratio obtained in the usual way, from zero current constant field fits to resting membrane potential data. Nor can they be reconstructed by summing the constant field current for potassium with that for any other single ion. They can be reconstructed, however, by summing the constant field current for potassium with a constant conductance component. It is concluded that the leak current and the resting membrane potential, therefore, are determined by multiple ionic components, at least three and possibly many. Arguments are presented suggesting that ion permeability ratios obtained in the usual way, by fitting the constant field equation to resting membrane potential data should be viewed with skepticism.

**INTRODUCTION**

For most operations on voltage-clamp data, complete extraction of ion specific currents requires an estimate of the leak current. Hodgkin and Huxley (1952) and Frankenhaeuser and Huxley (1964) took the leak current-voltage characteristic as linear for squid and node. However, Adelman and Taylor (1961) observed a rectifying leak current-voltage characteristic for squid. Similar observations have been made in giant squid (*Dosidicus*) axons (E. Rojas, personal
communication). For Myxicola axons steady-state leak conductance is not constant when examined under hyperpolarizing pulses (Binstock and Goldman, 1969).

This paper describes our detailed analysis of leak current in Myxicola giant axons. Rectification similar to that reported by Adelman and Taylor was observed. The experimental data have been compared to various simple theoretical models and a satisfactory quantitative description of the current-voltage characteristic has been obtained.

A preliminary report of some of these results has been made (Binstock and Goldman, 1969).

METHODS

All methods are as previously described (Binstock and Goldman, 1969; Goldman and Binstock, 1969). Leak current measures are confined entirely to early times; i.e., times similar to the time of peak of the transient current. For hyperpolarizing clamp pulses, current falls off, generally exponentially, in time to a steady-state value, and the steady-state leak current-voltage curve is not congruent with that for the early leak. Similar findings have been reported for squid (Moore et al., 1967) and Homarus (Narahashi and Haas, 1968). Steady-state leak could have been computed using the method of Moore et al. However, such computations might just as well be left to such a time as there are experimental data for comparison, e.g. on tetraethylammonium chloride injection (Armstrong and Binstock, 1965). In the work presented here leak current ($I_L$) always means early leak.

RESULTS

In six axons the transient current reversal potential, ($E_{Na}$), determined by the reversal method (Goldman and Binstock, 1969), was compared to that determined by a leak subtraction method. The leak estimates were obtained by pairing each depolarizing clamp step near $E_{Na}$ with an identical hyperpolarizing step. The currents at each hyperpolarizing step were read at the time of peak transient current of the corresponding depolarizing step. $E_{Na}$ was then taken as that potential at which the difference between the leak and peak transient currents was zero. In every case $E_{Na}$ as determined by the reversal method was significantly larger than that estimated from leak subtraction, the mean difference being 12.4 mv with a range from 5.5 to 15.5 mv. As the reversal method seems to be highly reliable (Goldman and Binstock, 1969), these results by themselves indicate that the leak current-voltage relation, $I_L(V)$, is clearly not symmetrical around the holding potential but rectifies in a manner similar to that reported for the squid (Adelman and Taylor, 1961).

Direct measures of $I_L(V)$ can be obtained by recording in tetrodotoxin (TTX). At a TTX concentration of $1 \times 10^{-7}$ m or greater there is no inward current (Binstock and Goldman, 1969). For depolarizing pulses $I_L$ is taken
at the minimum in the current record (Binstock and Goldman, 1969 b, Fig. 4, bottom). Currents in response to hyperpolarizing steps are read at the same time as the corresponding depolarizing pulse. Two representative $I_L(V)$ curves are shown in Fig. 1. The solid curves are fit by eye to the points. Each of six axons examined in this way showed clear rectification. As may be seen in Fig. 1, for *Myxicola*, taking $I_L(V)$ as symmetrical around the holding potential or the equivalent for values near $E_{Na}$, taking the conductance measured with large hyperpolarizing pulses as constant, constitutes a particularly poor operation for obtaining leak corrections. In Fig. 1, a leak estimate, indicated by a dashed line, was made assuming that the conductance obtained for large hyperpolarizing pulses is constant.

Note that constant resting conductance (Fig. 1, dotted line) is not a bad approximation over most of the range of depolarizing pulses tested (approximately to $E_{Na}$). Fig. 1 B shows a particularly favorable case and Fig. 1 A a more typical one. Near $E_{Na}$ the constant resting conductance estimate is less than $I_L$ by about 25% (0.03–0.04 ma/cm²) on the average, compared to a mean of almost 50% for the type of operation illustrated by the dashed line. This procedure can be substantially improved upon (see Discussion). However, for most purposes, i.e. unless some determination like very accurate (1–2 mv) estimates of $E_{Na}$ is required, the constant resting conductance method of estimating $I_L$ should produce an adequate approximation.

Note that $I_L$ is defined here in an entirely operational sense, i.e.; it is that correction on the transient membrane current needed in order to obtain the component carried entirely by sodium ions. In the Hodgkin and Huxley (1952) formulation (see also Cole and Moore, 1960) $I_L$ defined in this way contains a contribution from the specific delayed current. Such contributions

![Figure 1](image-url)
should be reduced to a minimum with hyperpolarizing prepulses (Cole and Moore, 1960). Fig. 2 shows the $I_L(V)$ for two axons with (solid circles) and without (open circles) prepulses. A hyperpolarizing prepulse of 40 mv and 30 msec produces only a 10–20% reduction in $I_L$ for large depolarizing pulses, indicating that any such contribution from the delayed current component is small (see particularly Fig. 2 A). Also the origin of this prepulse effect is not really clear. It may be due to a reduction in delayed current as noted above. However, in several axons (e.g. as seen in Fig. 2 B) a small but clear negative conductance appeared on prepulsing indicating the activation of some residual sodium conductance. In any case it is only necessary for the subsequent analysis (see Discussion) to insure that $I_L(V)$ as defined here does not contain a significant contribution from the specific transient or delayed current components.

**Figure 2.** Prepulsed (●) and nonprepulsed (○) leak current-voltage relations in *Myxicola* giant axons. Prepulses were −40 mv for 30 msec. A, axon 68M76, B, axon 68M66. Note the compressed current scale in A relative to B.

**Discussion**

With a measure of $I_L(V)$ a convenient practical operation on the transient current which will produce a satisfactory approximation to the sodium component may be selected. For *Myxicola* axons, constant resting leak conductance is a fair approximation, especially for moderate command pulses; i.e., up to about +70 to +80 mv.

A second point of interest in these data is the properties of the leak current itself. For the analysis presented here, the measured $I_L$ is assumed to be essentially uncontaminated with either specific transient or delayed current components; i.e., to be a passive system. The first question that may be asked is whether the leak rectification is of a simple Nernst-Planck type; i.e., may be computed from the constant field equation (Goldman, 1943; Hodgkin and...
Katz, 1949). The smooth curves of Fig. 3 have been drawn according to

\[ I_L = P_J \frac{F^2 V}{RT} \left( \frac{[J]_o/[J]_i}{(1 - e^{FV/RT})} \right), \tag{1} \]

the Goldman-Hodgkin-Katz equation, where \([J]_o, [J]_i\) are the external and internal activities, \(P_J\) is the membrane permeability to ion \(J\), \(V\) is membrane potential, and \(R, T, F\) have their usual significance. \([J]_o/[J]_i\) is determined by the zero current potential. The holding potential in these experiments is always the natural resting potential. The dotted curve is arbitrarily normalized at \(-100\) mv. The dashed curve is computed with \((P_J[J]_o)_{\text{outward}}/(P_J[J]_i)_{\text{inward}} = 0.5\).

In no case can \(I_L(V)\) be fit by a single constant field equation. This, of course, is as expected. In Myxicola, as is generally true for excitable cells, the resting membrane potential is largely determined by the distribution of potassium ions (Goldman, 1968). However, the resting membrane potential in ASW is about 20 mv depolarized to the potassium equilibrium potential. This requires that more than one ion carry the resting membrane current. If \(I_L\) is really uncontaminated by the specific transient and steady-state currents, then the analysis of \(I_L(V)\) is really an analysis of the system determining the resting

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure3.png}
\caption{Leak current-voltage relation in a Myxicola giant axon. Dotted curve, constant field prediction normalized to \(-100\) mv. Dashed curve, constant field prediction with \((P_J[J]_o)_{\text{outward}}/(P_J[J]_i)_{\text{inward}} = 0.5\).}
\end{figure}
membrane potential, now expanded beyond the zero current point. The results shown in Fig. 3 only illustrate that no single component; i.e., neither Cl nor K alone carries the $I_L$.

Note that both $I_L$ observed as a response to depolarizing pulses and $I_L$ observed as a response to hyperpolarizing pulses may each be fairly well fit by a constant field type equation, but with different values for $P_i[J_o]$. This suggests that outward and inward $I_L$ are carried largely by different ions. For the six axons tested $(P_i[J_o])_{outward}/(P_i[J_o])_{inward}$ needed to fit the entire curve was remarkably uniform, ranging from 0.45 to 0.55. These values may be used to improve somewhat on the constant resting conductance method of correcting for $I_L$. However, the procedure is cumbersome and the constants, $P_i$, $[J_o]$, no longer have a well-defined physical interpretation.

We expect, therefore, that $I_L(V)$ is actually the summation of several ionic components, each showing constant field type rectification; i.e.,

$$I_L(V) = I_K(V) + \sum I_i(V).$$  

However, Goldman (1968) could well describe the resting membrane potential vs. $[K_o]$ data in Myxicola axons by

$$V = \frac{RT}{F} \log \frac{[K_o] + P_{Na}/P_K [Na_o]}{[K]_\infty + P_{Na}/P_K [Na]},$$

the Goldman-Hodgkin-Katz equation at zero membrane current. This requires

$$I_L(V) = I_K(V) + I_{Na}(V).$$

Attempts were made, therefore, to reconstruct $I_L(V)$ according to equation (4). $I_K$ and $I_{Na}$ were both computed from the constant field equation (1). $[K]_\infty$ was taken as 340 mM (Goldman, 1968), and $P_K$ was determined for each axon by taking $I_L$ at $E_{Na} = I_K$. $[Na]$ was taken as 21.4 mM (Goldman and Binstock, 1969) and $P_{Na} = 0.03 P_K$ (Goldman, 1968). The $[Na]$ value used by Goldman was substantially higher than the value used here. However, this will change the value of $P_{Na}/P_K$ computed from equation (3) by less than 0.5%. Note that this procedure introduces no arbitrary constants.

Fig. 4 shows a typical result of such computations. In no case could equation (4) be used to reconstruct $I_L(V)$; nor can the fit be improved by selecting either $P_K$ or $P_{Na}/P_K$ arbitrarily. Increasing $I_{Na}$, by increasing $P_{Na}/P_K$, sufficiently to produce a good fit for large hyperpolarizing steps, will produce a large discrepancy at the holding potential and for moderate depolarizing steps. Also, $P_K$ cannot be increased sufficiently to improve the fit for large hyperpolarizing pulses without introducing a substantial discrepancy for large depolarizing pulses, as $I_{Na}$ must be close to zero in this range.
Clearly, various applications of the constant field equation to membrane current and potential data (i.e. equations (3) and (4)) do not produce a self-consistent set of results. This is perhaps not very surprising when one considers that the resting membrane potential is independent of \([\text{Na}]_o\) (Goldman, 1968; Goldman and Binstock, 1969) contra equation (3). It is very likely, therefore, that the constants, i.e. \(P_{\text{Na}}/P_{\text{K}}\), derived from the usual application of the constant field equation to resting membrane potential data, do not have the physical significance usually attributed to them; i.e., the resting \(I_K\) is balanced by some current other than or in addition to \(I_{\text{Na}}\).

Note in Fig. 4 that the fit of the reconstructed \(I_L(V)\) to the experimental points is extremely good near the resting potential. In no case (six axons) was the deviation of calculated from experimental results any worse than this, confirming that \(P_{\text{Na}}/P_{\text{K}} = 0.03\) gives a good fit for the resting membrane potential. It is only for large displacements from the holding potential that it becomes evident that the leak current (and the resting membrane potential) is not determined solely by the distribution of sodium and potassium ions. It is necessary therefore, to view ion permeability ratios computed from resting membrane potential data alone, with considerable skepticism.

It should be emphasized that these conclusions do not in any way reflect on the validity of the zero current case of the constant field equation, but rather on the usual interpretation given to a common application to experimental data. Indeed, for monovalent cations, for example, equation (3) may be obtained by a number of methods other than the assumption of constant field. (See Goldman (1968) for a summary of some of the relevant theoretical literature.)
Considerable insight may still be gained into the \( I_L \) process if, failing to account for \( I_L \) in terms of \( I_K \) and \( I_NA \) only, it may be accounted for entirely by \( I_K \) and some other single ion current; i.e.,

\[
I_L(V) = I_K(V) + I_1(V). \tag{5}
\]

This assumption was tested in the following way. \( I_K \) was computed from the constant field equation as described above. However, \( P_K \) was selected arbitrarily. For each \( P_K \) selected the computed \( I_K(V) \) was subtracted from the experimental \( I_L(V) \). Where \( I_K \) equals \( I_L \), \( I_1 \) equals 0. This defines \( [i]/[i]. \) At \( E_K \), \( I_L \) equals \( I_1 \). This defines \( P_1[i]. \) This procedure was repeated for 5% increments in \( P_K \) over a range of \( P_K \) values sufficient to attribute from 10–

100% of the outward \( I_L \) to \( I_K \). Note that this procedure requires only a single arbitrary constant.

Of the six axons for which such computations were made, Fig. 5 shows a particularly successful reconstruction. Clearly equation (5) is not adequate to describe \( I_L(V) \). These results indicate that \( I_L \) is carried by multiple, i.e. at least three, ionic components. The procedure described above could be expanded to include an additional component, but this would require three arbitrary constants. A more useful procedure is illustrated in Fig. 6. The solid curves in Fig. 6 are drawn according to

\[
I_L(V) = I_K(V) + G_m V_c \tag{6}
\]

where \( I_K(V) \) is again computed from the constant field equation, \( V_c \) is the command voltage, and \( G_m \) is a conductance. In every case equation (6) describes the data extremely well. For these reconstructions two arbitrary con-
constants are required, $P_K$ and $G_m$. The values used in the reconstructions are listed in Table I. These data, of course, could have been fit equally well, using Goldman’s (1968) $P_{Na}/P_K$ value, with an expression of the form

$$I_L(V) = I_K(V) + I_{Na}(V) + G_m V_e$$

(Fig. 4), but as the $G_m V_e$ component does not have a well-defined physical interpretation, nothing new is learned. Note (Fig. 2) that the shape of the prepulsed $I_L(V)$ is generally similar to that of the nonprepulsed. All the computations presented here, actually on nonprepulsed data, would produce identical results, except for the exact value of the constants listed in Table I, if carried out on prepulsed data.

Various physical interpretations may be given to a linear current-voltage characteristic (see e.g., Woodbury, 1969). However, one attractive possibility is that the constant conductance component is itself the summation of a number of ionic currents, each showing constant field rectification and which are simply well-approximated by a constant conductance over the range of

\[ \begin{array}{ccc}
\text{Axon} & P_K & G_m \\
68M65 & 6.80 \times 10^{-7} & 0.430 \\
68M66 & 7.90 \times 10^{-7} & 0.608 \\
68M69 & 7.45 \times 10^{-7} & 0.520 \\
68M71 & 6.90 \times 10^{-7} & 0.516 \\
68M76 & 28.8 \times 10^{-7} & 0.407 \\
68M77 & 8.25 \times 10^{-7} & 0.613 \\
\end{array} \]
values examined here. Each one of these components would actually constitute some part of that portion of the resting current usually attributed entirely to $I_{na}$. At rest the current carried by any individual component, i.e. sodium, chloride, calcium, or internal ions may be very small indeed. Of course, this view may be directly tested under internal perfusion.

The conclusions developed here may be summarized as follows. The $I_e$ in *Myxicola* is carried by multiple; i.e., at least three ionic components. The $I_e(V)$ may be reconstructed by the summation of a potassium ion component showing constant field type rectification, and a constant conductance component. Ion permeability ratios obtained in the usual way from the effects of ion substitutions on the resting membrane potential may have a very ambiguous physical meaning.

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