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REVERSAL POTENTIALS CORRESPONDING TO MECHANICAL STIMULATION AND LEAKAGE CURRENT IN *MYXICOLA* GIANT AXONS

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The response of a *Myxicola infundibulum* giant axon to a transverse mechanical stimulus is an increase in membrane conductance. The similarity of the reversal potential for this conductance increase and the reversal potential for leakage current, together with other similarities, suggest similar pathways for these two processes. Depolarization of the reversal potential with increased mechanical stimulus is best explained in terms of a gradual change in the mechanically-stimulated ionic pathways.

Julian and Goldman [1] demonstrated that mechanical stimulation of a lobster axon causes an increase in sodium permeability. In order to further characterize this type of conductance increase, we have applied mechanical stimulation to a *Myxicola* axon under voltage-clamp conditions

By employing a constant mechanical stimulation over a range of membrane potentials, it is possible to determine the relevant reversal potential corresponding to the mechanically-induced conductance increase. The reversal potential obtained in this way is a measure of the selectivity of the ionic pathway corresponding to the mechanical stimulation. An important question regarding this pathway is whether it is a channel with two discrete conductance states, similar to those that have been found for sodium and potassium channels in axons, or whether its conductance changes gradually in response to mechanical stimulation. A general property of all discrete channels is that they have unique reversal potentials. In order to test whether the mechanically-induced pathway conforms to this criterion, we have varied the magnitude of the mechanical stimulus to determine whether the reversal potential is constant.

Another question regarding the mechanically-induced pathway is whether it is related to the ionic leakage that occurs in the absence of mechanical stimulation. We have performed a test related to this question by measuring the reversal potential for leakage in the same *Myxicola* axonal preparation.

Myxicola infundibulum were obtained from Maritime Research Associates, Deer Island, NB. Experiments were performed on intact axons of approx. 0.5 mm in diameter by conventional voltage clamp techniques. Methods for preparing and voltage clamping the axon were as described by Binstock and Goldman [2,3].

Mechanical stimuli were supplied to the axon from above by the movement of a loudspeaker driven by a power amplifier and controlled by a pulse generator. The loudspeaker was coupled to the axon by means of a thin metal rod connected to a plastic stylus

2 mm in diameter. This assembly was positioned just above the voltage sensor of the internal electrode of the voltage clamp. Movement of the stylus interrupted the signal between a light-emitting diode and a photoresistor so that the output of the photoresistor monitored the position of the stylus The rise time of the stylus movement was about 1 ms.

The composition of the artificial sea water was. 340 mM NaCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 105 mM Tris (hydroxymethyl) aminomethane, pH 7.4. High K solutions were prepared by an equivalent per equivalent substitution of NaCl with KCl and tetraethylammonium (Eastman Kodak Co , Rochester, NY) was substituted for Tris The holding potential was always at the natural resting potential in each solution.

The change in membrane potential induced by a mechanical stimulus depends on both the magnitude and the rate of change of the stimulus. Following a subthreshold stimulus, there is a long phase of depolarization, sometimes preceded by a brief hyperpolarization. The depolarization decays exponentially with a time constant of 1–10 min. When the depolarization reaches a threshold value, an action potential is initiated

In order to test whether the mechanically-induced depolarization involves the same type of sodium channels responsible for the rising phase of the action potential, we added tetrodotoxin, which is known to block these sodium channels, to the external solution. This eliminated the action potential, but did not affect the mechanically-induced depolarization This behavior is similar to that observed in the Pacinian corpuscle and the crayfish stretch receptor [4], and indicates that the sodium channels responsible for the action potential are not involved in the mechanical transduction.

In order to separate the primary effect of the mechanical stimulation from the secondary effect of induced depolarization, we measured the response to the mechanical stimulus under voltage clamp. There is considerable question about the adequacy of our voltage clamp, since the mechanical stimulus probably affects different parts of the membrane within the clamp region very differently. This spatial inhomogeneity no doubt distorts the measured currents. Therefore, we regard our voltage clamp membrane currents in general as rough approximations, and consider quantitatively only the case where the mechanically induced component of the membrane current is zero. For this condition, the distortion should be small, and should not significantly influence the reversal potential (the membrane potential at which the membrane current changes sign). Fortunately, the reversal potential provides a very useful measure of membrane selectivity.

When the axon was clamped to its resting potential, the current response to a 5 ms mechanical stimulus was an inward current of long duration with an exponential decay time constant of 10 s. This response under voltage clamp shows that mechanical stimulation causes a change in the conductance of the axonal membranes. Probably because of the variability of the amount of connective tissue associated with each axon, the dependence of current on stylus displacement was found to vary from axon to axon. However, for any given axon, the measured current is a monotonic function of displacement.

If a second stimulus was applied shortly after the first stimulus, it elicited a current of much smaller amplitude. As the time between stimuli was increased, the amplitude of the second current gradually approached the amplitude of the first current. This refractoriness has a time constant of about 3 min Thus, it was necessary to wait about 10 min between pulses in order to obtain reproducible results. Whatever the reason for the long refractoriness of the *Myxicola* axon, it presents a serious methodological obstacle, limiting experimentation on a single axon to not more than about 20 mechanical stimuli.

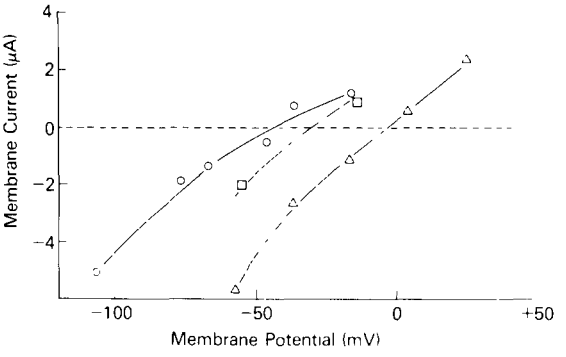

Fig. 1. Peak change in current as a function of membrane potential for three different mechanical stimulus amplitudes 29 μm (circles), 53 μm (triangles), and 60 μm (squares). Stimulus duration 5 ms

In order to obtain current-voltage curves corresponding to mechanical stimulation, we clamped the axon to each desired voltage, waited until steady state was reached, and then applied a mechanical stimulus. For each curve, the amplitude of the mechanical stimulus was kept constant and the change in peak membrane current corresponding to a given voltage was recorded. Also, we waited at least 10 min between pulses. The results are shown in Fig. 1. The stimulus in the first run (circles) was 29 μm, and the corresponding reversal potential was −46 mV. For the second run (triangles), the stimulus was increased to 53 μm, and the corresponding reversal potential was −3 mV. Movement of the reversal potential in the depolarizing direction in response to an increase in stimulus amplitude was experimentally confirmed in three other axons. In order to rule out the possibility that this effect was caused by deterioration of the axon, we made a third run (squares) with the axon of Fig. 1 after the axon resting potential had declined to −42 mV and the action potential amplitude to 80 mV. For this deteriorated axon, the reversal potential for a mechanical stimulus of 60 μm was −30 mV, indicating that the damage that occurred to the axon caused the reversal potential to move in the hyperpolarizing direction. Thus, any component of the original change of reversal potential from −46 mV to −3 mV that might have been caused by axon deterioration would have gone in the hyperpolarizing direction, and the actual movement in the depolarizing direction must have been caused by the increase in the amplitude of mechanical stimulation.

The apparent reversal potential for leakage currents (V_L) was determined under voltage clamp in the presence of 10^{-6} M tetrodotoxin (Sigma Co., St. Louis, MO) to eliminate all of the sodium currents and 100 mM tetraethylammonium to eliminate the majority of the potassium currents. Leakage currents are not measurably affected by tetraethylammonium or tetrodotoxin [5,6]. Applied externally, 100 mM tetraethylammonium was found to eliminate approximately 80% of the potassium currents in *Myxicola* [7]. The steady-state current-voltage curves at different time intervals are shown in Fig. 2. The mean resting potential and the reversal potential (V_L) for six different axons in artificial sea water are shown in Table I. V_L was taken as the membrane potential at which the leakage current (I_L) changed sign. From

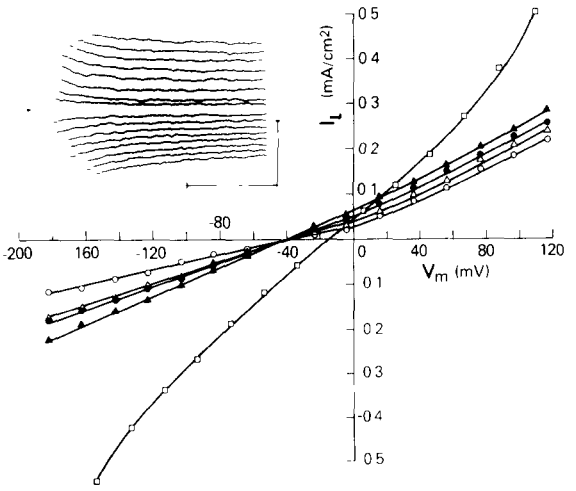

Fig. 2. Leakage currents I_L plotted as a function of membrane potential V_m for the same *Myxicola* axon at different time intervals: 0 min (○———○), 60 min (△———△); 120 min (●———●), 180 min (▲———▲). Same axon was deliberately damaged with a large and long depolarizing pulse (□———□). Fig. 2 (Inset). Families of voltage-clamped leakage currents I_L associated with eight step depolarizations and eight step hyperpolarizations in increments of 20 mV from the holding potential of −44 mV Scale. 0 2 mA/cm², 0.5 ms. Temperature· 8°C.

Fig. 2 (inset), it can be seen that as long as I_L is measured after the capacitive transient, the exact time of measurement is not important in determining a unique V_L. Table I shows that in artificial sea water, V_L is about −43 mV (28 mV depolarized with respect to the resting potential).

In *Myxicola,* the resting potential in artificial sea water is depolarized approximately 20 mV with respect to the potassium equilibrium potential [8] Addition of tetrodotoxin to block the sodium current

TABLE I

APPARENT REVERSAL POTENTIALS OF LEAKAGE CURRENT FOR *MYXICOLA* AXONS

$[K]_0$	Resting potential (mV)	V_L (mV)	n
0	−71.0 ± 5.5	−43.0 ± 3.6	6
100	−24 0 ± 1.1	−16.3 ± 1.2	3
440	14.7 ± 1.5	7.0 ± 2.0	3

(I_{Na}) has no effect on the resting membrane potential. The resting potassium current (I_K) is therefore balanced by some current other than I_{Na}. This implies that I_L is non-zero, albeit small, at rest. Fig. 2 shows that I_L corresponds to an approximately 25 $\mu A/cm^2$ inward current in artificial sea water.

Values of V_L similar to those measured under voltage clamp and shown in Table I were obtained when we simply monitored the resting membrane potential as I_{Na} and I_K were eliminated by drugs. In artificial sea water, for a mean resting potential of -67 ± 6.7 mV ($n = 6$), V_L was found by this method to be $-42\ 3 \pm 3.8$ mV ($n = 6$)

When the external K^+ concentration, $[K]_0$, was increased, the resting potential depolarized. V_L was found to move towards zero relative to the resting potential (Table I), regardless of the new resting membrane potential reached. The change in membrane potential in the presence of tetraethylammonium is completely reversible in low $[K]_0$ and is generally reversible in high $[K]_0$ as long as the axon did not stay depolarized for more than 30 min. Also the amount of tetraethylammonium used, beyond 100 mM, does not seem to affect V_L. This would indicate that contamination of I_L by residual I_K is probably quite small.

Very little is known about the pathways or ions responsible for leakage currents. If the leakage pathways have a higher selectivity for potassium than for other cations, then the shift in V_L in high $[K]_0$ could be wholly or partially explained by the change in external potassium concentration. However, changes in V_L can also be caused by a gradual increase in the size as well as number of existing pathways

In general, I_L has been found to increase as a function of time but no report has been made as to what happens to V_L under similar conditions We have found that as long as the resting membrane potential did not change by more than a few mV during the course of an experiment, V_L remains essentially unchanged (Fig. 2), although I_L increased slightly in amplitude and the rectification became less as a function of time At the end of one experiment we deliberately damaged the axon by a large and long depolarizing pulse. Under this condition, V_L was found to move in a depolarizing direction towards zero potential (Fig. 2), probably reflecting a reduced ion selectivity with damage.

From our current-voltage measurements between 4 and 23°C, we found that the magnitude of I_L has an average Q_{10} of 1.63 ± 0.16 ($n = 12$), with larger Q_{10} for membrane potentials depolarized (1.87) than hyperpolarized (1 31) from rest. By comparison, Schauf [9] reported a Q_{10} for leakage current of 1.34 ± 0.09, but did not specify the membrane potentials. Although the magnitude of the leakage current is clearly temperature-dependent, the leakage reversal potential does not depend upon temperature.

There is a substantial change in the reversal potential of the mechanically-induced conductance when the amplitude of the mechanical stimulus is changed. This would not be expected for the type of two-state ionic channels that have been observed in biological membranes. Two-state channels are either off or on When they are off, they do not contribute to the membrane conductance or reversal potential, and when they are on, their selectivity properties correspond to a unique reversal potential.

A change in reversal potential could occur if there were two different types of mechanically-stimulated ionic channels with different reversal potentials. If the channels with the more hyperpolarized reversal potential were preferentially excited by small mechanical stimuli, and the channels with the more depolarized reversal potential were preferentially stimulated by large mechanical stimuli, then increasing the stimulus amplitude would cause the reversal potential to become more depolarized. Although this two-channel hypothesis could explain the observed change in reversal potential, it would also predict two kinetic components for the decay of the mechanically-stimulated conductance. Since this decay seems to have only one kinetic component, the two-channel hypothesis is unlikely. A more likely explanation for the change in reversal potential is that the ionic pathways gradually change in response to the stretching of the membrane caused by mechanical stimulation.

What is the conductance of the mechanically-induced ionic pathways in the absence of mechanical stimulation? As shown in Fig. 2, there is a component of membrane conductance that is not associated with sodium or potassium channels This leakage component could well be the conductance of the mechanically-stimulated pathways when there is no mechanical stimulation. In support of this hypothesis, neither conductance can be blocked by a specific drug, sug-

gesting that they may both be nonspecific pathways, rather than distinct channels.

Our measurements of the reversal potential of the leakage pathway and of the small-stimulus mechanically-stimulated pathway give values that are very close, –43 and –46 mV, respectively. This provides additional support to the hypothesis that mechanical stimulation opens further the ionic pathway that conducts leakage current. The non-zero reversal potentials indicate that the pathways have a selectivity for certain ions. The values of the reversal potentials are consistent with sodium and potassium as the ions responsible. However, confirmation of the specific ions involved will require further experiments in which the external and internal ionic concentrations are systematically varied.

The leakage current density in biological membranes is several orders of magnitude larger than the leakage current density in lipid bilayer membranes [10]. These two types of membranes also differ in that biological membranes contain a large amount of membrane protein. Therefore, we suggest that membrane proteins in general, rather than specific channel proteins, provide the ionic pathways for leakage current. This could occur, for example, if the membrane proteins reoriented some of the lipids to form intermittent or stable polar pathways for ionic transport. The mechanically-stimulated conductance could arise from an increase in the average number of such nonspecific pathways, and the change in reversal potential could arise from an increase in their average size.

There are several applications of an understanding of mechanical transduction in axons. One is to compare and contrast it with mechanical transduction in stretch receptors. Another is its possible relevance to an understanding of the influence of stretch on electrical conductance and metabolic activity in muscle [11,12].

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