BRIEF COMMUNICATION

CURRENTS RECORDED THROUGH SMALL AREAS OF SQUID AXON MEMBRANE WITH AN INTERNAL VIRTUAL GROUND VOLTAGE CLAMP

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ABSTRACT A new voltage-clamp apparatus for the squid axon has been implemented to enable recording of currents through small areas of axon membrane. The performance of this clamp was tested by recording total sodium currents from perfused axons (I_{total}) and sodium currents from small membrane patches (I_{patch}), which were recorded from inside the axon with an L-shaped pipette. The I_{patch} records, although four orders of magnitude smaller than I_{total}, were stable and showed normal kinetics and voltage dependence, and appeared to reflect the activation of a small population of normal sodium channels. The size of the current recorded from the patch was mainly a function of the tip diameter of the L-shaped pipette and of the shunt resistance between inside the pipette and the axoplasm.

In studying current fluctuations related to ionic channels in excitable membranes, it is advantageous to isolate and record from a very small membrane patch (Fishman, 1975; Neher et al., 1978). Reducing the recording area enhances channel-related fluctuations relative to noise from other sources. Several techniques have recently been developed for electrical isolation of small patches. Successful application of these techniques in squid giant axons will make it possible to apply the ensemble method for measuring current fluctuations (Sigworth, 1977), and to record current from single membrane channels (Conti and Neher, 1980; Sigworth and Neher, 1980).

We report here the general features as well as the performance of a voltage-clamp technique for the squid giant axon which can be used to record current through small areas of membrane. 30-mm-long segments of axon were placed in a chamber and internally perfused. The main features of the voltage-clamp circuit are illustrated in Fig. 1. Internal voltage (V_{in}) was recorded through a 0.6 M KCl-filled pipette (*1*). Vin was fed back to an internal control amplifier (ICA) which held V_{in} at ground potential by passing the appropriate current through a platinized-platinum wire (*3*) placed inside the axon. External voltage (V_{out}) was recorded through a silver-silver chloride electrode (*2*). V_{out}-V_{in} was fed to an amplifier (ECA) which controlled membrane voltage by varying the bath potential relative to internal ground through two large platinum electrodes (*4*) placed longitudinally along the outside of the axon. Voltage-clamp command pulses were generated from a PDP-8 computer (Digital Equipment

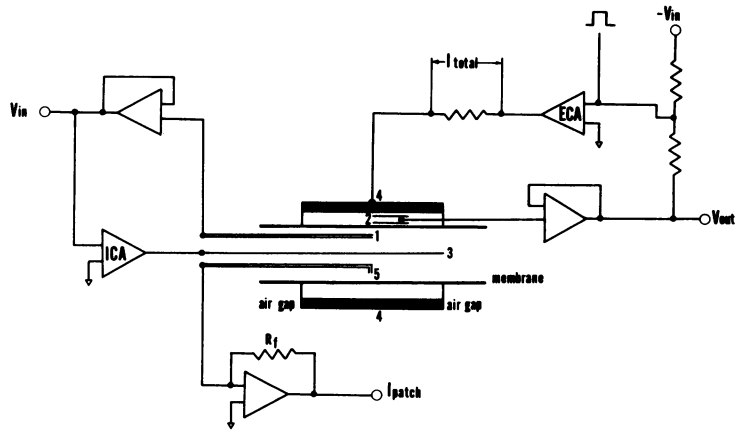

FIGURE 1 Voltage clamp and current recording circuit. The numbers in the diagram refer to the following electrodes: *1*, internal voltage pipette; *2*, external voltage electrode; *3*, internal platinized-platinum axial current passing wire; *4*, external platinized-platinum electrodes; and *5*, L-shaped patch pipette. The large external electrodes (*4*) were 15 mm long and extended the entire length of the axon between the air gaps, so I_{total} was recorded from this length of axon.

Corp., Maynard, Mass.) through a D/A converter and fed to the summing junction of ECA. The current generated by the whole axon (I_{total}) was monitored by recording the voltage drop across a 100 Ω resistor connected in series with the output of ECA.

Currents through small patches of membrane were recorded simultaneously with I_{total} using a method similar to the one described by Conti and Neher (1980). The method involves placing an L-shaped pipette inside the axon and maneuvering the tip to the inside surface of the membrane. The bent part of the pipette was 100–200 μm in length and had an outside diameter of 70–100 μm. As the pipette tip was fire polished the inside diameter decreased to a final diameter of 10–25 μm. This patch pipette (*5*) was connected to an operational amplifier wired as a current-to-voltage converter to measure current through the pipette. The particular patch pipette used in the experiments reported here had an inside tip diameter of 20–25 μm and a DC resistance of ~0.5 MΩ. A floating 25-μm Diam platinum wire was placed inside the pipette to decrease impedance and thereby minimize series resistance errors caused by patch electrode resistance. The patch pipettes were filled with a solution containing 150 mM tetramethylammonium (TMA) glutamate, 50 mM TMA fluoride, 20 mM sodium glutamate, 10 mM Tris and 450 mM sucrose. All records were obtained at 8°C. Linear leakage and capacitative currents were subtracted out electronically using the P/4 technique (Armstrong and Bezanilla, 1974).

For our purposes the major advantage that this particular voltage-clamp design offers over the more commonly used design (Cole and Moore, 1960; Hodgkin et al., 1952) is the maintenance of the interior of the axon at virtual ground potential, which eliminates capacitative currents across the wall of the patch pipette thereby improving the frequency response. Clamping the axoplasm to virtual ground was also done in voltage-clamp techniques developed for the node of Ranvier (Dodge and Frankenhaeuser, 1958; Nonner, 1969) and suggested for the squid giant axon by Levis (1979).

The performance of this voltage-clamp was assessed by comparing currents recorded from

the whole membrane (I_{total}) with current generated by the patch (I_{patch}). Although the general characteristics of I_{total} are similar to sodium currents recorded with the more usual voltage-clamp configuration, our chamber lacked guard electrodes; total membrane currents recorded with this clamp are therefore not highly accurate, since the ends of the preparation are not well controlled. To record accurate total currents, the measuring region could be restricted to a central region and guard electrodes in the periphery could be driven by a separate control amplifier.

Fig. 2 *A* shows superimposed sodium currents, recorded as I_{total} from 15 mm of axon, generated by voltage-clamp steps from −80 mV to the indicated voltages. The current-voltage relationship from these records is shown by the filled symbols in Fig. 2 *C*. Patch recordings of sodium currents from the same axon are shown in Fig. 2 *B*, and their current-voltage relationship is plotted as the open symbols in Fig. 2 *C*. The important point to note is that the kinetics and voltage dependence of these currents indicate that they were generated by a normal population of sodium channels, and the amplitude of the currents suggests that the number of channels was small. Previous attempts to record patch currents from the inside of the axon upon steps in membrane voltage have failed (Conti and Neher, 1980). Conti and Neher refer to the appearance of large and slow artifacts that contaminate their current records.

Although the general shape and position of the *I-V* curves are similar there are several apparent differences between the sets of currents shown in Fig. 2. First, the peak-to-steady-state sodium current ratio is noticeably smaller for I_{total} than for I_{patch}. Second, the reversal potential for the I_{total} current is ~12 mV less positive than for I_{patch}. We feel that the patch records are a more accurate representation of the membrane sodium currents for the

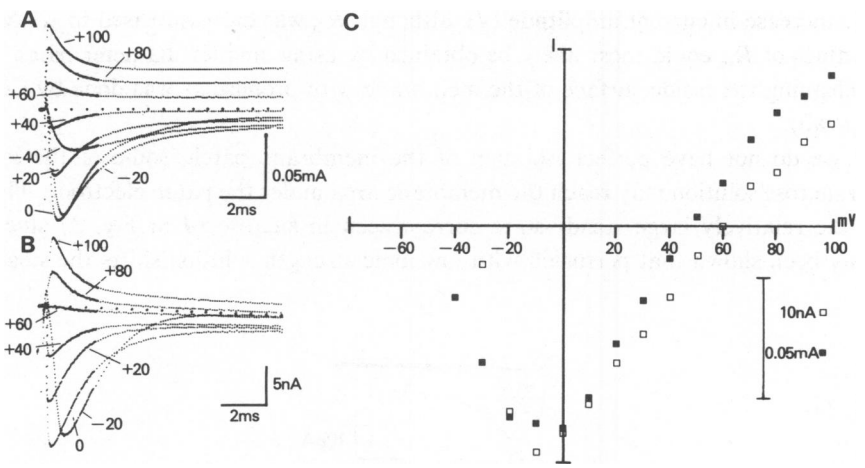

FIGURE 2 *A*, Na currents (I_{total}) generated by 10-ms voltage-clamp pulses to the indicated voltages from a holding potential of −80 mV. This axon was ~440 μm in diameter so that the total area recorded from was approximately 0.21 cm^2. *B*, Na currents through a 25 μm (inside tip diameter) patch electrode generated by 10-ms voltage clamp pulses to the indicated voltages from a holding potential of −80 mV. Solutions for *A* and *B* were the same. External solution: 225 mM NaCl, 50 mM $CaCl_2$ and 240 mM Tris 7.0. Internal solution: 150 mM TMA glutamate, 50 mM TMA fluoride, 20 mM Na glutamate, 10 mM Tris 7.0, and 540 mM sucrose. *C*, Current-voltage relationships for peak currents from the records shown in *A* (■) and *B* (□).

following reasons. Uncorrected I_{total} records were found to have a much higher leakage current than the patch records and this leak presumably flowed out the cut ends of the axon. If this current increases nonlinearly with voltage, due in some unknown way to the damaged ends of the axon, it could produce a large steady-state current and an apparently lower reversal potential. The reversal potential predicted by the Nerst equation is 58.8 mV, very close to the reversal potential of the patch records.

The patch currents shown in Fig. 2 *B* were recorded with a 25-μm tip-Diam pipette that was touching lightly against the membrane. With this large diameter pipette it is impossible to obtain the high shunt resistances (R_{sh}) which can be obtained with smaller diameter pipettes (cf. Neher et al., 1978). Therefore, the I_{patch} records are not an accurate reflection of the total current generated by the patch, since much of this current apparently flows through R_{sh}. The I_{patch} current generated at 0 mV represents a current density of ~3.7 mA/cm^2 assuming the area recorded from was 490 μm^2 (i.e. a circle of 25 μm Diam). Since the I_{total} current at 0 mV was ~0.42 mA/cm^2, the I_{patch} records must have been recorded from a much larger area than the 25-μm Diam circle we assumed.

An improvement in the isolation of the membrane patch can be obtained under the appropriate conditions and Fig. 3 illustrates the technique we have used. The four currents illustrated are patch recordings for voltage steps to 0 mV from a holding potential of −80 mV. *1* was obtained with the pipette placed in the center of the axon and the tip ~100 μm away from the membrane. The pipette was then moved until it just touched the membrane and the current was found to increase appreciably (*2*). An even higher current amplitude could be obtained by pressing the pipette tip more firmly against the membrane (*3*), which presumably increases R_{sh}. The internal perfusion of the axon was then switched to a low ionic strength solution (without changing the solution in the current recording pipette) and there was a dramatic increase in current amplitude (*4*), although R_{sh} was only increased to ~1 MΩ. Still higher values of R_{sh} could most likely be obtained by using smaller diameter patch pipettes and by cleaning the inside surface of the membrane with pronase as was done by Conti and Neher (1980).

Since we do not have perfect isolation of the membrane patch, some of the low ionic strength sucrose solution may reach the membrane area under the patch electrode. This could explain the relatively large steady-state current seen in sucrose (*4* in Fig. 3) since it has previously been shown that perfusion with low ionic strength solution shifts the steady-state

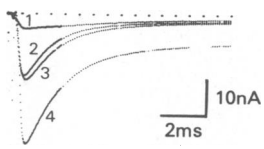

FIGURE 3 Na currents through a 25-μm Diam patch electrode as the shunt resistance from pipette interior to axon interior increased. Numbers refer to successive 10-ms voltage steps to 0 mV from a holding potential of −80 mV. Records *1–3* were recorded with the same solutions as in Fig. 2 and the pipette in the following positions: *1*, center of the axon; *2*, just touching the axon membrane; and *3*, pressing against the membrane. *4* was recorded after switching to an internal solution of the following composition: 850 mM sucrose, 50 mM TMA fluoride, and 10 mM Tris 7.0.

inactivation curve to more positive voltages (Chandler et al., 1965; Moore et al., 1964). Therefore, in the sucrose solution inactivation may be less complete at 0 mV, resulting in more steady-state sodium current. A second possibility is that there is a change in the junction potential at the tip of the internal voltage electrode when the internal perfusion is switched to low ionic strength solution. Therefore, the true membrane potential in this solution may be at a voltage where inactivation is less complete.

The major point of this communication is that the sodium current records presented illustrate the ability of our virtual ground voltage-clamp configuration to produce stable sodium currents whose amplitude is limited mainly by the size of the pipette tip and by the value of the shunt resistance. Since the internal virtual ground circuit effectively eliminates the stray capacitance across the wall of the patch pipette, the frequency response of the recording system is improved. This may be why we have not seen the long lasting artifacts observed by Conti and Neher (1980) upon step changes in membrane voltage. By using smaller diameter patch pipettes, we should be able to achieve acceptable values of R_{sh} so that this voltage-clamp configuration will be useful for recording membrane current fluctuations and estimating single channel conductance (Sigworth, 1977). In addition, it should be possible to record the current through single membrane channels.

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