BRIEF COMMUNICATION

FREQUENCY DOMAIN ANALYSIS OF ASYMMETRY CURRENT IN SQUID AXON MEMBRANE

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ABSTRACT The change in capacity of squid axon membrane during hyper- and depolarizations was investigated in the absence of ionic currents after the membrane was treated with pronase. In the presence of the inactivation process (h parameter), failure to observe the gating current in the frequency domain was attributed to the rapid attenuation of the possible capacity change during depolarizations, which is likely to be due to the sodium activation process. Elimination of the h process would therefore enable us to observe the gating current in the frequency domain as the change in the capacitance component of membrane admittance. However, even after the inactivation process was abolished by pronase, the capacity of the axon membrane remained constant when ionic currents were blocked by external tetrodotoxin and internal Cs^+ ion. Actually capacity was observed to decrease slightly with depolarization, contrary to the prediction based on the magnitude of gating currents.

Gating currents through the nerve membrane are measured by suppressing or blocking all ionic currents after subtracting the linear capacitive current ($c_m \cdot dV/dt$ term) (1–3). Since gating currents are measured as the difference between capacitive currents during depolarizing and hyperpolarizing pulses, they may be called more generally "asymmetry currents." In essence, gating currents are time-dependent displacement currents that are nonlinear with applied voltages.

Since the gating current is a displacement current measured in the time domain, its behavior implies that the capacity, if measured in the frequency domain, will change under the same conditions. R. E. Taylor (personal communication) and Armstrong and Bezanilla (4) estimated the possible increase in the capacitance to be about $0.35\,\mu F/cm^2$ with a pulse of 40–60 mV based on the magnitude of gating currents. Since gating currents are nonlinear phenomena, these calculations, which seem to be based on the linear transformation from the time domain to frequency domain, may not be rigorous. Nevertheless, these calculations are helpful in estimating the magnitude of expected capacitance increase with depolarization of axon membranes.

Takashima (5), and Takashima et al. (6) made a series of experiments to investigate

with an AC admittance bridge (B-221, Wayne Kerr Lab Ltd., Chessington, Surrey, England) the change in membrane capacitance of squid axons with depolarizations in the presence and absence of ionic currents. They reported a substantial increase in capacity, i.e., from 0.95 to 1.25 $\mu F/cm^2$ between -120 and $+100$ mV membrane potential in the presence of sodium as well as potassium currents. However, they also reported that the change in capacitance was nearly completely abolished by blocking ionic currents with tetrodotoxin (TTX) in the bathing solution and impermeable Cs ion in the axoplasm. Fishman et al. (7) measured admittance changes of squid axon membrane using a pseudo-random noise method under similar conditions and reported a minute decrease in the capacitance with depolarizations.

In the experiments performed by Fishman et al., the membrane was depolarized by a long pulse having a duration of 1 s, and Takashima et al. used square pulses 20 ms long. Under either of these circumstances it is possible that the capacitance change, most likely to be due to sodium channels, may have been inactivated before the completion of the measurement. This is particularly true in the case of Fishman et al.'s experiment because of their use of long pulses.

In the present work attempts were made to detect the increase in the capacitance by abolishing the sodium inactivation process ("*h*" process) so that the capacitance change due to the sodium activation will not fade with time. In these experiments, axons were internally perfused with pronase (0.2 mg/ml) in the presence of tetraethylammonium (TEA) (15 mM). The destruction of *h* process can be easily confirmed by voltage clamp experiments (see Fig. 1).

Under these circumstances, the potassium currents as well as the sodium inactivation processes are effectively eliminated and the possible admittance change during depolarization or action potential must be due to the sodium activation process alone. The procedure of transient admittance change measurements during depolarization of the membrane was described in detail in previous papers (5,6). Therefore, it suffices to state here that a Wayne Kerr B-221 bridge combined with a PAR-TM124 lock-in amplifier (Princeton Applied Research, Princeton, N.J.) are used for these measurements. Fig. 2 shows the change in the membrane admittance during the prolonged

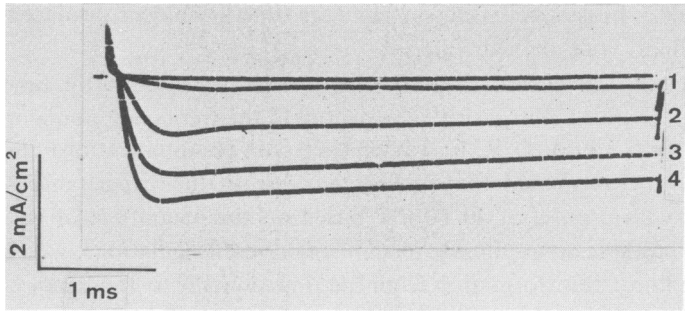

FIGURE 1 Voltage clamp diagram of axon membrane treated with pronase internal perfusion solution (0.2 mg/ml). Curves 1, 2, 3, and 4 are obtained with 20, 40, 50, and 60 mV depolarizations. Time and current scales are 1 ms and 2 mA/cm^2, respectively. Temperature, 10°C.

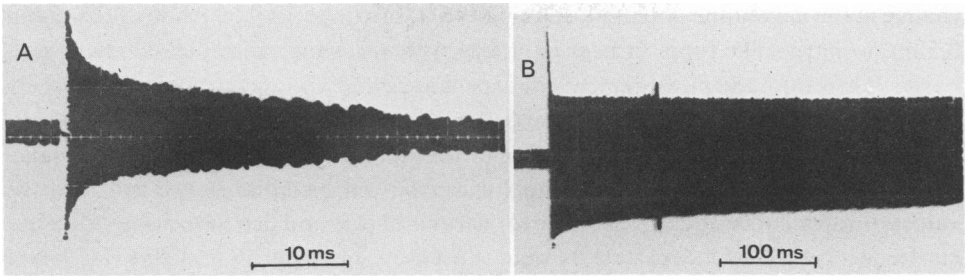

FIGURE 2 A. Admittance change during the action potential in the presence of 15 mM TEA in the axon. Note the attenuation of admittance change with time due to the inactivation process. Time scale, 10 ms. The duration of the action potential is 70 ms. B. Nonattenuating admittance change during the action potential in the presence of 15 mM TEA in the axon after the destruction of the inactivation process by pronase. Time scale, 100 ms. The duration of the action potential is about 3 s.

action potentials before and after the pronase treatment of the membrane (Fig. 2 A and B, respectively). Note that the admittance change before the elimination of *h* process attenuates with time while the abolition of the inactivation process causes a nonattenuating admittance change. The admittance changes are, in both cases, mostly capacitive and the conductance changes are relatively small. This indicates that the large conductance increase observed by Cole and Curtis during the action potential (8) arises mainly from the potassium activation process.

After these steps, TTX (3×10^{-7} M) is added in the external solution and sodium currents are blocked. The admittance change during a depolarizing pulse in the absence of all three ionic currents is shown in Fig. 3. To detect the small admittance change during depolarization and hyperpolarization, the gain of the lock-in amplifier was increased as much as possible. This is the reason why the oscilloscope trace (upper trace) is somewhat noisier than those of Fig. 2. The solid line (lower trace) is the

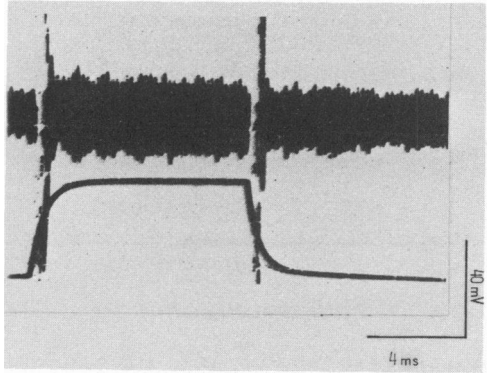

FIGURE 3 Upper trace, admittance change during a depolarizing pulse (80 mV 10 ms) after addition of TTX (3×10^{-7} M) with internal TEA (15 mM) after pronase treatment. Lower trace is change in membrane potential. The scale is only for the lower trace.

change in the membrane potential. During the transient period, which lasts for about 0.5 ms, a sharp spike (upper trace) interferes with the admittance measurement seriously. Therefore, the measurements of capacitance and conductance changes during pulses begin with a delay of 0.5–0.7 ms after the onset of pulses. As shown by the upper trace of Fig. 3, a slight perturbation of the AC bridge is observed. By rebalancing the bridge to the depolarization or hyperpolarized membranes, we can read the values of capacitance and conductance for various hyper- and depolarizations at different frequencies. Our measurements were carried out between 0.7 and 20 kHz. Since the time constant of gating currents at this potential is about 0.2 ms (3), the possible change in capacitance should be observed in the frequency range 1–5 kHz. The values of capacity at 1.5 kHz at various depolarizations are shown in Fig. 4. In addition, the change in membrane conductivity is also plotted. This figure clearly demonstrates a small increase in conductance and a small decrease in capacitance with the depolarization of the membranes instead of an increase in capacitance predicted from the value of the gating current. This observation is in agreement with those by Fishman et al. (7), who also reported a small decrease in capacitance and an increase in conductance with depolarizations.

In the previous experiments, one reason for the unsuccessful attempt to observe

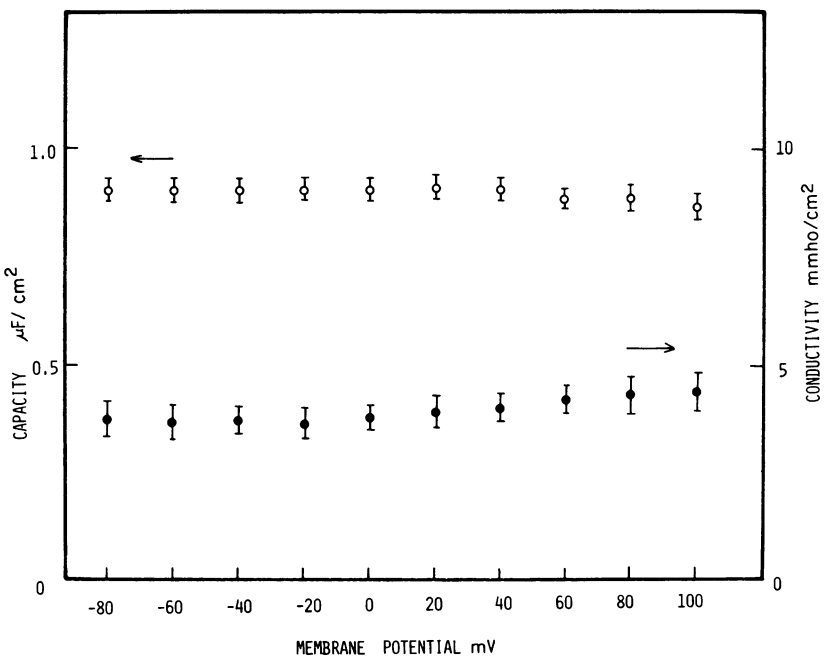

FIGURE 4 Changes in capacity and conductivity at 1.5 kHz with membrane potential. Left ordinate for capacity (open circles) and right ordinate for conductivity (closed circles). Statistical analyses are based on nine successful experiments in which deterioration of membranes was not noticeable during the measurement. These are values after the correction for series resistance.

changes in capacitance due to gating was the possible inactivation of the sodium channels. In the present experiment, the inactivation h process was abolished by the pronase treatment and therefore our result can no longer be attributed to the fast inactivation of the m process. Thus one of the explanations for the discrepancies between the time domain and frequency domain measurements is eliminated.

The magnitude of gating currents is on the order of 20–60 $\mu A/cm^2$ and is not easy to measure accurately in the time domain without resorting to a technique of signal enhancement. However, the capacitance change of 0.35 $\mu F/cm^2$ is not difficult to detect with AC bridges, which usually have sub-picofarad resolution. Although the transient measurements with an AC bridge are somewhat more difficult than static measurements, it is quite unlikely that the capacitance change of 0.3–0.4 $\mu F/cm^2$ was overlooked by our system and also by Fishman's pseudo-random noise technique. Therefore, the discrepancy between the time domain and frequency domain analyses remains unsolved. A clear separation of conductance and capacitive currents is sometimes difficult in the time domain analysis. Under these circumstances, it is possible that asymmetry currents observed in recent years are at least partially due to some phenomenon other than gating.

The author is indebted to Dr. H. M. Fishman for his stimulating discussion and to Dr. F. Bezanilla for his helpful advice.

The author is partially supported by ONR-N00014-76-C-642.

Received for publication 23 September 1977.

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