BRIEF COMMUNICATION

CURRENT PULSE-INDUCED VOLTAGE VARIATIONS IN BILAYER MEMBRANES

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ABSTRACT A current pulse apparatus is described that can charge bilayer membranes from an external potential under conditions so that the resulting membrane voltage variations can be recorded with ~100-ns resolution.

INTRODUCTION

Bilayer membranes, first prepared by Mueller et al. (1), have provided an impetus for membrane studies, since these model membranes are readily prepared in apparatus that makes them accessible for physical measurements. The development of electronic apparatus for making such measurements has been an integral part of membrane studies. The action of uncouplers of oxidative phosphorylation, for example, was initially studied in bilayer membranes with direct current resistance measurements (2–4). Subsequently, voltage pulse (5–7) and charge pulse (8,9) methods were used which enabled the kinetics of uncoupler transport to be studied. The resolution of charge pulse apparatus varies from ~0.2 to 10 μs (10–13). A progression to high speed measurements has also occurred in the study of photoelectric effects in bilayer membranes (14–19), where the resolution approaches 10 ns (20).

An electrical equivalent circuit of a bilayer membrane, cell, and associated apparatus is illustrated in Fig. 1*A*. Numerical values for the circuit components when using a 10-ml Teflon (Chemware, Chemplast Inc., Wayne, N.J.) cup, 1-M NaCl solutions, and a 1.2-mm diam bilayer membrane in a 1.5-mm diam hole in the cup are: C_c = 50 pF, C_m = 5 nF, and $2R_a$ = 200 Ω. With 1 M NaCl agar electrodes (20), $2R_e$ = 200 Ω. An external voltage applied through the electrodes and switch will charge C_c with a time constant of $\sim 2R_eC_c$. The membrane capacitance C_m will then be charged through $2R_a$ with a time constant of $2R_aC_m$. This time constant limits the speed of voltage clamped circuits, since the feedback current cannot complete charging the membrane capacitance until $\gtrsim 2R_aC_m$ has passed. A charge pulse delivered instantaneously through the electrodes will develop a voltage on C_c before the charge can pass through $2R_a$ to C_m. This voltage will be about C_m/C_c times the voltage ultimately obtained on the membrane. Charge pulse circuits will not complete charging C_m until the voltage on C_c and any external and stray capacitances have fallen to the voltage on C_m. Furthermore, the membrane voltage cannot be detected during this period because

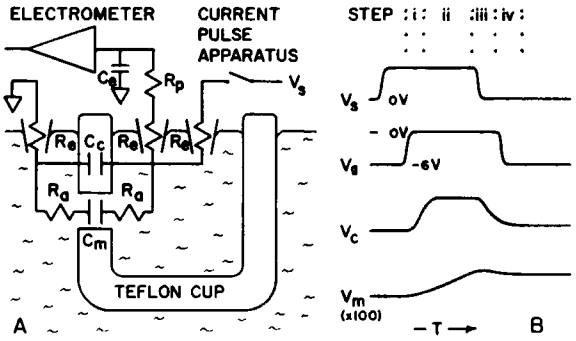

FIGURE 1 (*A*) The equivalent circuit for the membrane, cup, electrodes, and current pulse apparatus. Conductance paths with resistance values $\gtrsim 10^9$ Ω are ignored. (*B*) Voltage versus time during the operation of the current pulse apparatus. The steps are described in the text. V_g is the voltage applied to the FET gate input. V_c and V_m are the voltage across the cup and membrane, respectively.

electrometers detect the voltage on C_c (20). Consequently, fast voltage clamped circuits may ring, producing biphasic responses, while charge pulse circuits produce initial voltage spikes that decay exponentially.

These difficulties can be curcumvented by charging membranes with the current pulse apparatus illustrated schematically in Fig. 1*A*. It operates in four steps: (*i*) the transistor switch is closed, charging C_c to V_s. (*ii*) Charge from C_c and the switch flows onto C_m for a time T. (*iii*) V_s is set to zero, discharging C_c back through the switch. (*iv*) The switch is opened. The resulting voltage variations are illustrated in Fig. 1*B* for a typical case.

METHODS

A detailed schematic of the current pulse apparatus is given in Fig. 2. The electrometer, timing circuits, and faraday cage housing this apparatus are described elsewhere (20). Time delays within the current pulse apparatus are provided by AND gate signal propagation delays. R_p reduces the electrometer output ringing and slows its resolution to ~60 ns (Fig. 3*A*). See reference 20 for a discussion of factors limiting the electrometer resolution.

The higher speed membrane voltage traces (50 and 100 ns per division) were recorded by photographing the electrometer output on a Tektronix 5444 dual beam oscilloscope (Tektronix, Inc.,

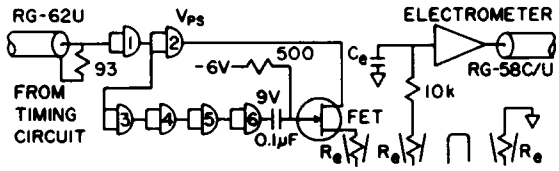

FIGURE 2 A detailed schematic of the current pulse apparatus, which was built on a 4 cm by 5 cm circuit board and mounted inside the electrometer box (20). Three DIP (dual-in-line package integrated circuits) quad TTL AND gates (type 7408, # 276-1822, Radio Shack) were used. AND gates 1, 3, 4, and 5 are single gates on one DIP. AND gates 2 and 6 are each four gates on the other two DIPs, with the four gates operating in parallel to lower the output impedance. Numbers above the AND gates give power supply voltages when they differ from 5 V. V_{ps} is varied through the use of batteries and switches in a battery box adjacent to the electrometer box. The FET is Motorola 2N3971. One electrode is soldered directly to the FET. The resistors are ½ watt, 5% carbon composition.

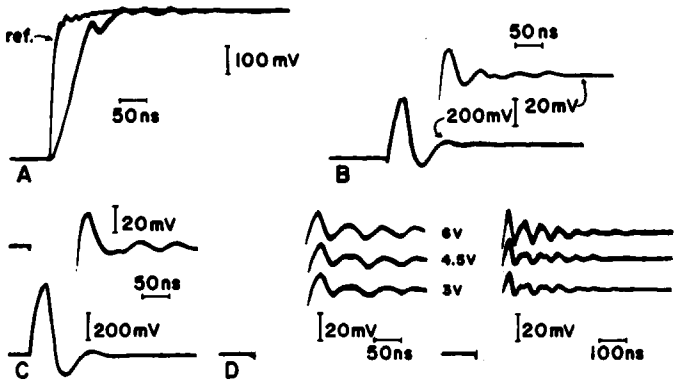

FIGURE 3 (*A*) Voltage waveforms illustrating the dynamic performance of the electrode-electrometer-oscilloscope system with R_p = 10 k Ω. The reference trace (ref) was generated by a photocell from an 8-ns laser light flash, with the photocell connected directly (via coaxial cable) to one oscilloscope vertical amplifier. The other trace was generated by a second photocell mounted in a dummy membrane cell. The traces were recorded simultaneously with beam splitters providing the same light intensity versus time to both photocells. (*B*) Voltage variations resulting from a current pulse (T = 40 ns, V_{ps} = 4.5 *V*) applied to a dummy membrane cell. Traces were recorded at two different amplifier gain settings. At the higher gain setting, the trace was either off-scale or too faint to be photographed for ~90 ns. (*C*) Voltage variations resulting from a current pulse (T = 40 ns, V_{ps} = 6 *V*) applied to a regular membrane cell with no membrane present. The traces were recorded at two different gain settings. (*D*) Voltage variations resulting from a bilayer membrane and 40-ns current pulses with V_{ps} = 3, 4.5, and 6 *V*, respectively.

Beaverton, Oreg.) by opening the oscilloscope camera shutter and then running the trace. Lower speed traces (≥1 μs per division) were stored on a Tektronix 5113-03 storage oscilloscope and then photographed. The initial voltage spike seen in the 1-μs per division trace of Fig. 4*C* is an artifact produced by the limited storage oscilloscope amplifier bandwidth (~2 MHz) and the larger voltage excursion illustrated in Figs. 3*B* and *C*.

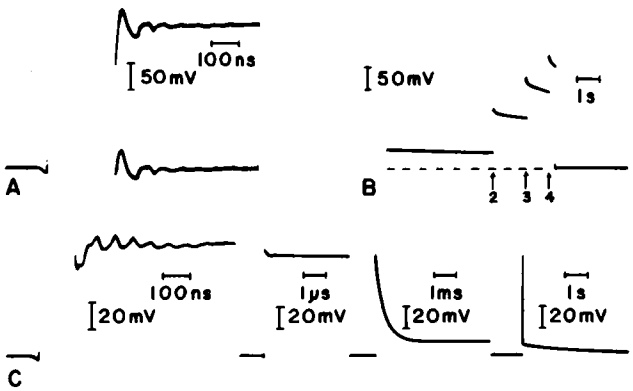

FIGURE 4 (*A*) Voltage variations resulting from a 200-ns current pulse (V_{ps} = 6 V) applied to a bilayer membrane. The lower trace was recorded with the same cell when no membrane was present. (*B*) Membrane voltage variations resulting from multiple current pulses (T = 200 ns, V_{ps} = 3 V). The first current pulse (not shown) was followed by three others, marked with vertical arrows numbered 2, 3, and 4. The membrane ruptured shortly after the fourth current pulse. (*C*) Membrane voltage variations resulting from a current pulse (T = 80 ns, V_{ps} = 6 V) applied to a bilayer membrane in the presence of 10^{-7} M sodium tetraphenylboron. (Photographs from the two oscilloscopes differ slightly in size, resulting in slightly different scale sizes on the high and low speed traces.)

A current pulse charges the membrane to a voltage V_m, where $V_m = Q_m/C_m \simeq (I\ T)/C_m \simeq (V_s\ T)/(2R_aC_m)$. V_m may be increased through V_s by increasing AND gate 2 power supply voltage (V_{ps} in Fig. 2), by increasing T in the timing circuit, or by decreasing C_m. C_m may be decreased by increasing the membrane torus area. Decreasing C_m by reducing the diameter of the hole in the Teflon cup results in an increased R_a value. The membrane voltage may also be increased by operating the current pulse apparatus several times in succession.

The bilayer membranes were prepared by the syringe method using 10 mg lipid/ml decane in apparatus described elsewhere (20). The lipid was phosphatidylethanolamine (p–3511, Sigma Chemical Co., St. Louis, Mo.). The membranes were prepared at room temperature (~23 C) in 1 M NaCl aqueous solutions in air, with 1 M NaCl agar electrodes (20). The sodium tetraphenylboron was obtained from J. T. Baker Chemical Co. (Phillipsburg, New Jersey).

RESULTS AND DISCUSSION

The current pulse apparatus performance has been evaluated in three arrangements: with a dummy membrane cell, with unmodified (high resistance) bilayer membranes, and with bilayer membranes in the presence of 10^{-7} M sodium tetraphenylboron. Voltage versus time recordings are given in Figs. 3 and 4.

The dummy membrane cell is a regular membrane cell that lacks a hole in the Teflon cup, where the membranes are usually formed, but it has a 5-nF capacitor cemented to the cup. The capacitor contacts the aqueous solutions through two Ag/AgCl electrodes, which have ~1.5 mm^2 surface area each. That is about the same area the bilayer membranes used have in contact with the aqueous solutions. The current pulse apparatus and electrometer contact the aqueous solutions in the dummy membrane cell through agar electrodes, as they do with regular membrane cells, so the dummy membrane cell provides an accurate mimic of a bilayer membrane, cell, and electrodes for evaluating the apparatus performance.

Current pulse apparatus with increased resolution should be possible, by using larger V_s values, which would allow T to be reduced without reducing V_m. A faster settling amplifier in the electrometer would also increase the resolution. Such amplifiers are now commercially available (see sales literature from Burr-Brown Research Corp., Tuscon, Az.). These changes should enable the resolution to approach the ~35-ns switching speed (close plus open) of junction field effect transistors (FETs). The open impedance of the FET is ~10^{11} Ω, so this current pulse apparatus does not significantly lower the observed membrane RC time. Insulating gate field effect transistors (IGFETs or MOSFETs) can switch faster than FETs, but their lower open impedance will lower the observed RC times for unmodified bilayer membranes.

The current pulse apparatus will allow high voltages to be used across membranes, without rupturing the membranes, as the switch can be closed a second time to discharge the membrane before it ruptures. This may be accomplished by substituting a two input OR gate for AND gate 5, with one OR gate input transmitting the pulse from AND gate 4, and the other OR gate input transmitting a new signal generated to close the switch a measured time interval after the current pulse. An operational amplifier used in place of AND gate 2 could also generate positive and negative current pulses by applying the signal pulses to the noninverting and inverting inputs of the operational amplifier. Alternating positive and negative current pulses may be of interest for studying dipole reorientations in membranes.

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