INSTANTANEOUS CURRENT-VOLTAGE RELATIONS DURING THE FROG VENTRICULAR
MYOCARDIAL ACTION POTENTIAL

YALE GOLDMAN AND MARTIN MORAD. DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF
PENNSYLVANIA SCHOOL OF MEDICINE, PHILADELPHIA, PENNSYLVANIA

The molecular mechanisms for the ionic fluxes which repolarize the
cardiac action potential are not known partly because a complete separation
of ionic currents has not been successful. In contrast to heart muscle
detailed evidence for the nerve membrane has accumulated which implies that
the ionic fluxes are independent and are driven solely by their individual
transmembrane electrochemical gradients.

To test the ionic independence hypothesis (Hodgkin, A.F. and Huxley, A.L.,
J. Physiol. 116:449-472, 1952) in heart muscle, we developed a new voltage
clamp technique (Goldman, Y. and Morad, M., Fed. Proc. 33:446, 1974) which
enables measurement of ionic current 2 to 5 msec after a rapid change in
membrane potential. This technique makes it possible to measure the true
membrane current and transmembrane potential without complications from
artifacts of extracellular leakage current, extracellular series resistance,
stray capacity or inhomogeneous distribution of potentials among various
cells in the preparation.

If the individual ionic channels are independent, the total membrane
ionic conductance should vary as permeabilities to Na^+, K^+, and Ca^{+2} change
during the action potential. To test this prediction, we have measured the
transmembrane current voltage (I-V) relation during the action potential of
frog (*Rana pipiens*) ventricular cells. The ionic current measured 5 msec
after application of voltage clamp pulses are plotted vs the test potential
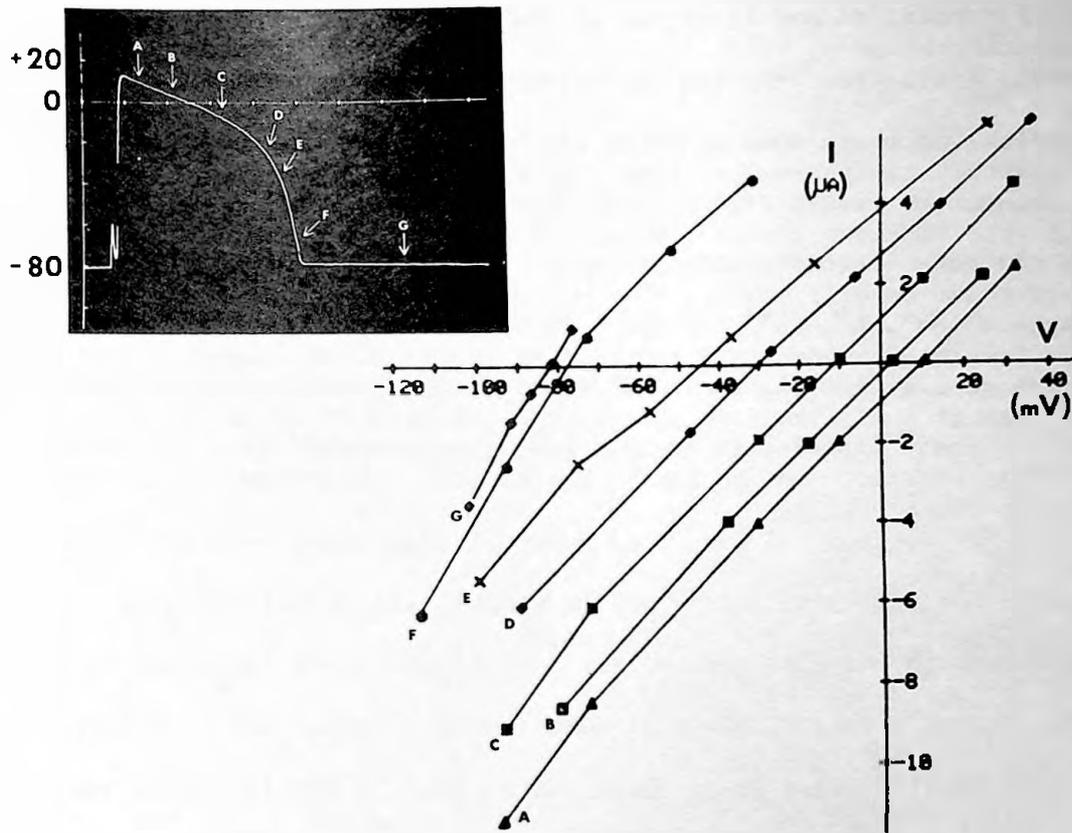
Figure 1: Current-voltage relations during various phases of the frog ventricular action potential. Clamp initiation time for each curve is indicated in the inset. Membrane conductance is constant during the plateau of the action potential. $[Ca^{++}] = 0.2$ mM.

In Figure 1. The different curves were obtained by initiating the clamps at various times during the plateau of the action potential (curves A-D), the rapid repolarization phase (curves E and F), and at rest (curve G). These current-voltage relations are considered to be "instantaneous" in the sense that little change in ionic permeabilities occurred between time of application of the test pulse and time of measurement of the ionic current (5 msec).

The I-V relations are linear and shift slowly upward and leftward during the plateau. The total membrane ionic conductance is given by the slope of

each I-V relation and is higher at rest (curve G), than during the plateau
(curves A-D). However, the conductance during the plateau and rapid re-
polarization phase down to about -50 mV is remarkably constant. This is
an unexpected result if the ionic channels are mutually independent because
the membrane ionic conductance is the sum of the individual ionic conduc-
tances of Na^+, K^+, Ca^{++} and Cl^-. As the permeabilities of these ions change
during repolarization, the total membrane conductance should change accord-
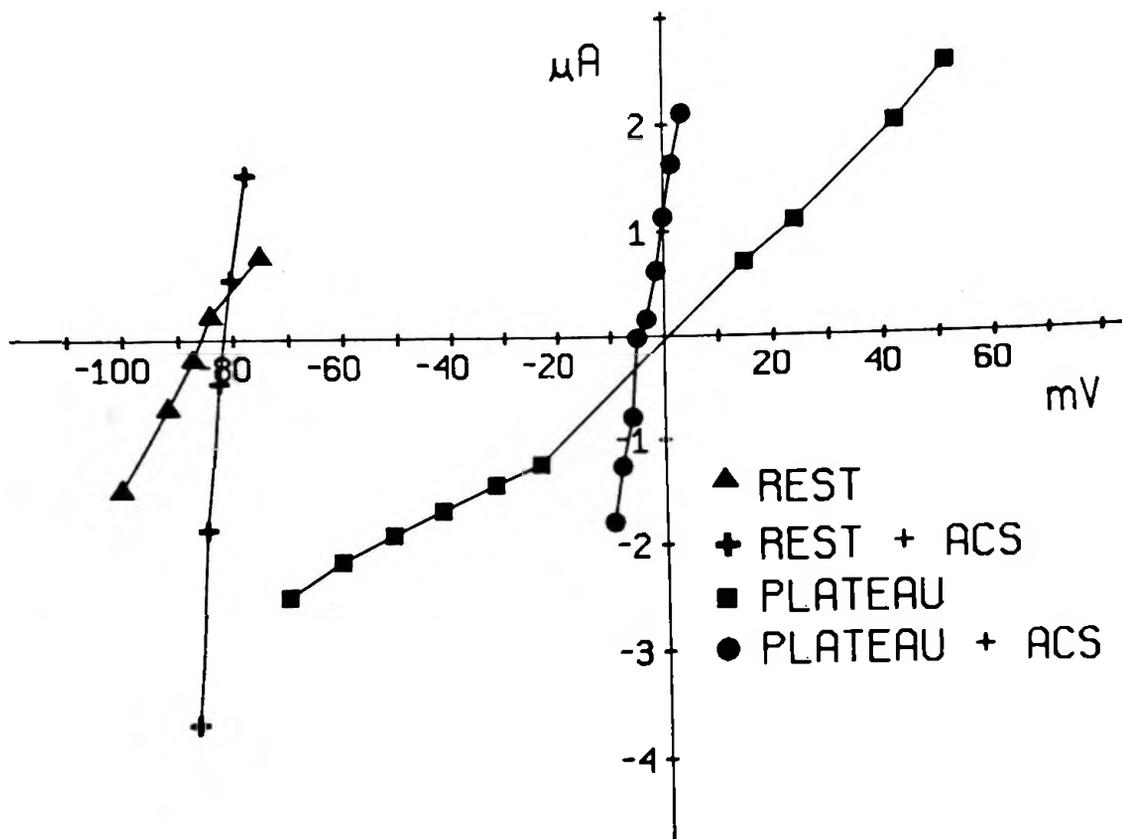ingly. These experiments suggest that the permeabilities of the physiological

Figure 2: Current-voltage relations in the absence and presence of
5 x 10⁻⁶M acetyl strophanthidin. AcS markedly increased
membrane conductance during plateau and rest.

Ions may be coupled In the membrane so that the total ionic conductance during the plateau Is constant.

Preliminary experiments have shown that this constant conductance phenomenon is sensitive to the activity of Ca^{++} in the external bathing medium and to agents which influence the Ca^{++} permeability of myocardial cells. For instance the digitalis analog acetyl strophanthidin (AcS) alters cellular calcium which results in potentiation of contractile force. AcS also inhibits the Na-K pump. The effect of AcS on the "instantaneous" I-V relation of the frog heart cell is shown in Figure 2. Current-voltage relations recorded during the plateau of the action potential and at rest are shown before and after addition of a high dose $(5 \times 10^{-6}M)$ AcS to the bathing medium. The membrane conductance Is markedly increased both during the action potential and at rest in the presence of AcS. This suggests that AcS has other surface membrane effects besides inhibition of the Na-K ATPase system and that these effects alter the coupling of ionic movements which results in constant ionic membrane conductance during the plateau. A multi-ionic carrier mechanism provides an attractive hypothesis to explain the constant conductance characteristic of the heart action potential (Morad, M. and Goldman, Y. Progr. Biophys. Mol. Biol. 27:257-313, 1973). Calcium ions might serve as a necessary agent for this coupling and may determine the stochiometry of the transport system.