# Effects of time-dependent electric fields on membrane transport

R. Dean Astumian
Departments of Surgery and of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois 60637 USA

How do time dependent electric fields influence membrane proteins? This question is central to many areas of biophysics including neurophysiology and "biomolecular electronics". It is also important for understanding whether and how very weak electric fields such as those associated with power lines and electric appliances can affect human health.

Horn, in a fascinating article in this issue (1), proposes a novel way by which the interaction between electric fields and membrane proteins can be used to help study the kinetics and mechanisms of membrane transport, and to determine which mechanistic steps in a reaction are likely to be electrically active. The crux of the method is to measure the average (or steady-state) rate of transport of an ion or molecule across a membrane as a function of the frequency of an applied weak oscillating electric field. The expected response can be described as a sum of Lorentzian curves (2, 3). The midpoint of each Lorentzian is the inverse relaxation time of one normal mode of the system. In this regard the proposed technique is very similar to stationary relaxation techniques (4), such as dielectric relaxation, or to ultrasonic absorption, in which the power absorbed from an oscillating perturbation is measured as a function of frequency. The neat thing about this method is that an average steady-state rate is determined instead of power absorption, which is experimentally very tricky to measure. This opens up the possibility of relaxation kinetic studies to experimentalists who have focussed previously on steady-state measurements. I anticipate that the technique will initially find widest applicability for kinetic studies of membrane transporters because of the relative ease with which an oscillating potential can be generated across a membrane.

One approach is to apply an external ac electric field between 1 and 100 V/cm to a suspension of cells. Because the membrane is much more resistive than either the intra- or extracellular medium, the applied field causes an oscillatory charge build up at the membrane interface, resulting in an ac membrane potential of 5–50 mV. The magnitude of the oscillating potential depends linearly on both the amplitude of the applied field, and on the radius of the cells being studied. The rise time is governed by the resistance and capacitance of the spherical membrane. For moderately sized cells ($<100$ $\mu$m radius) this is $\sim 10$ $\mu$s, so frequencies up to nearly a megahertz (somewhat higher if corrections are made) are accessible. These techniques have been used for studying the $Na^+K^+$ ATPase in erythrocytes (5), and an ATPase in carrot protoplasts (6, 7). Typical data, and the fit to theory, for the $Na^+K^+$ ATPase are shown in Fig. 1, *a* and *b*, for $Rb^+$ (an analogue of $K^+$) and $Na^+$ transport, respectively.

Another approach, which is the focus of the paper by Horn, involves studying individual cells mounted in a chamber such that the inside of the cell is exposed to one pool, and the outside to another pool of buffer solution, with the only electrical connection between the two pools provided by channels and transporters in the membrane. Similarly, the plane bilayer method may be useful for studying purified proteins in a controlled environment. An electric potential difference can be directly applied across the membrane by two electrodes. The rise time is governed by the electrode resistance and capacitance. For certain techniques, such as the double vaseline gap voltage clamp method (8), rise times as small as a few $\mu$s are achievable. For other techniques, such as patch or whole cell voltage clamp, it may be more difficult (though certainly not impossible) to push the electrode design to allow for frequencies greater than a KHz.

The frequency response method should be considered as a useful complement to other techniques. The main requirements are that an electric potential must be rapidly established across a membrane to allow for fairly high frequency studies, and that the resulting average flux must be measurable against the ambient background. The latter requirement suggests that radioactive tracer methods may be particularly useful, although situations in which optical dyes or direct measurement of the average electric current would be appropriate are easily imaginable. It should be remembered that the frequency response technique is just one way of determining relaxation times and amplitudes. These experimental quantities, no matter how obtained, are model independent parameters that partly characterize a system for given experimental conditions. As with any technique, further interpretation in terms of molecular structure or kinetic mechanism requires postulation of a model for which a theoretical response can be calculated and compared with the experimental response.

## Relevance for physiological behavior

In his paper, Horn stressed the possibility of using applied time-dependent fields to study the kinetics and mechanisms of membrane transport. The resulting theory, however, is also relevant to understanding the role of the membrane potential in governing the function of many membrane proteins (9, 10). The classical


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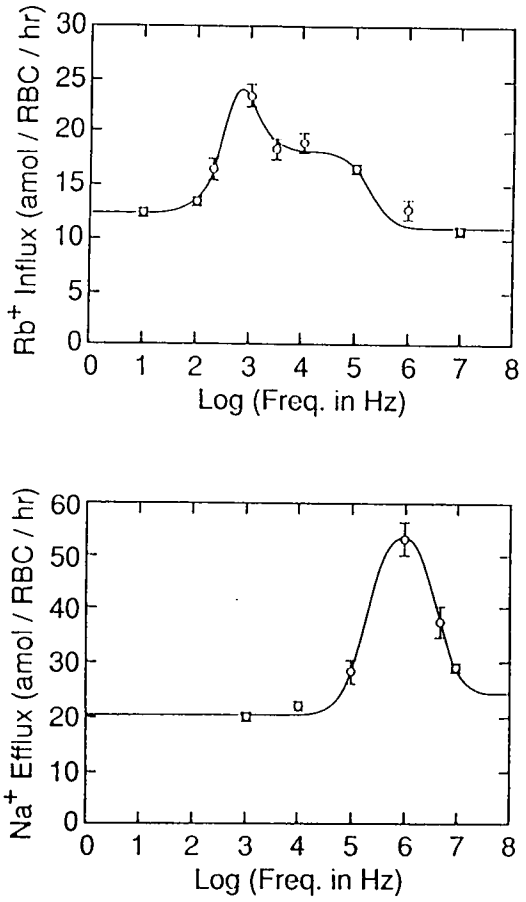


FIGURE 1 Theoretical frequency dependence of membrane transport flux compared with experiment. The upper curve is a sum of three Lorentzians ($\tau_1 = 282$, $\tau_2 = 265$, and $\tau_3 = 0.85$ $\mu$s) and the lower curve is a sum of two Lorentzians ($\tau_3 = 0.85$ $\mu$s, and $\tau_4 = 0.0371$ $\mu$s) with one characteristic frequency common to the two curves. These curves are fit simultaneously to $Rb^+$ and $Na^+$ flux measurements from reference 5. The bars on the data points represent one standard deviation uncertainty. Taken with permission from reference 3.

theory for steady-state enzyme kinetics makes use of many implicit assumptions, including the assumption that the thermodynamic parameters such as pressure, temperature, and electric field strength are approximately constant on a time scale long compared with any reaction under consideration. While this is usually valid for reactions carried out in vitro under carefully controlled conditions, it is often not reasonable under physiological circumstances particularly for membrane reactions in vivo. The membrane potential is not a static quantity. In many systems, the membrane potential undergoes regular oscillations (11), but even more commonly, the opening and closing of membrane channels causes fluctuations in the membrane potential (12). Combined with the realization that many if not most membrane proteins have transitions involving significant movement of charge (13, 14) (voltage gated channels and electron and ion translocating proteins come immediately to mind), it seems likely that many membrane processes are regulated by changing the frequency spectrum of the membrane potential. This conjecture has not been explicitly tested due to the difficulty of making simultaneous measurements of proteins kinetics and of the instantaneous membrane potential. Recent developments make such experiments possible, and I anticipate that much interesting information will be obtained by correlating membrane potential dynamics with the functional activity of membrane proteins.

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