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R. S. EISENBERG

Department of Physiology, Rush University, Chicago, Illinois 60612

ABSTRACT

Changes in ion concentration can be expected to accompany natural activity in any preparation with infolded membranes or narrow spaces between cells. Such preparations have complex electrical properties because some of the membranes are isolated by the resistance of the solution in narrow extracellular spaces. Analyses of the diffusional and electrical consequences of these structural complexities are intimately related: both require morphometric measurement and theoretical analysis of the structure. The measured properties of complex tissues, either diffusional or electrical, depend on the properties of many membranes and intra- and extracellular compartments. Fitting a structural theory to one type of experimental data separates some of the measured properties of the tissue into the properties of its components. Fitting electrical data alone gives many electrical parameters of the tissue components. Fitting diffusional data alone may give some of the permeability and diffusional parameters of the tissue components. A complete understanding of ion accumulation probably requires a concomitant analysis of electrical properties. —**Eisenberg, R. S.** Structural complexity, circuit models, and ion accumulation. *Federation Proc.* 39: 1540–1543; 1980.

Structural complexity characterizes the membrane systems of most tissues. Skeletal muscle fibers have tubular invaginations of the surface membrane (the T-system) that branch to surround the myofibrils and conduct the action potential from the surface into the depths of the fiber. Cardiac muscle fibers have tubules and/or narrow clefts between electrically coupled cells. Epithelia have a system of narrow, lateral intercellular spaces important in the transport of fluids and solute. The lens of the eye is formed by fibers tightly packed, electrically coupled, but with a significant extracellular space between the cells. The glial space of the central nervous system is riddled with narrow intercellular clefts. Even axons have some properties produced by the small extracellular space in the immediately surrounding sheath of Schwann cells.

Only cells that float free in the blood or lymph can be expected to have outer membranes without infolding or other anatomical complexity.

The structural complexity of tissues is often directly involved in their function and is usually involved in the interpretation of measurements from that tissue. Considerable attention has been paid to the electrical properties associated with structural complexity. Circuit models are available to describe the pattern of current flow in a number of tissues (see a forthcoming review (9) of the work started by Falk and Fatt (10)). Some attention has also been paid to the linkage between structural complexity, complex electrical properties, and ion accumulation for the classical preparation of frog skeletal muscle (3–5, 14, 17). But the *obligatory* relationship between diffusional, electrical, and morphological properties is perhaps not as widely understood as it should be.

This paper outlines the steps used to analyze the electrical properties produced by structural complexity. It seems likely that a quantitative analysis of ion accumulation requires essentially the same steps, since the structural and physical processes underlying electrical and diffusional processes are so similar. Most of the effects of complex structure are produced by the narrow, tortuous extracellular spaces between cells or within tubules. These spaces introduce significant resistance to current flow, they produce significant impediment to diffusion, and they thereby produce significant change in ionic and solute concentrations during natural activity. The physical and morphological parameters determining diffusional and electrical properties of narrow extracellular spaces are closely related, and often identical. Thus, the analysis of electrical complexity—the circuit model of a complex tissue—is intimately related to the analysis of ionic concentration changes induced by that complexity.

MORPHOLOGICAL CONSIDERATIONS

It seems obvious that analysis of the effects of structural complexity must start with the structure. Qualitative and quantitative descriptions of the structure are required since both the form and the amount of the various structures have important effects on physiological properties. In the past, less attention has been paid to the structure than is logically appropriate, perhaps because of the difficulties that were once associated with morpho-

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metric analysis. But these difficulties have been reduced to manageable proportions in many laboratories. Thin sectioning and marking of extracellular spaces have been used extensively, for example, in analysis of the T-system of skeletal muscle. Thick sectioning and observation in the high-voltage electron microscope have also been used to study skeletal muscle (see, for example, Peachey and Eisenberg, 18). Most importantly, the stereological techniques reviewed by Weibel (20) are widely known and have been extended by Eisenberg, Kuda, and Peter (7) to oriented tissues.

The limiting factors in morphological analysis are now the sheer work involved and the difficulties inherent in tissue preservation. The work involved should not be minimized: it is often necessary to study a tissue under many functional and experimental conditions. Each condition must be expected to produce changes in the size of structures and perhaps their connections, particularly in the size of clefts and tubules. Therefore, the analysis of each physiological condition requires a morphometric analysis of the structure in that condition.

The difficulties in tissue preservation also should not be minimized. Much work is under way to make techniques of preservation more reliable, but that work is beyond the scope of this review. Suffice it to say that the reliability of new techniques—rapid freezing for example—must be established by comparisons of size and shape *in preserved and living tissue*, not by arguments or other forms of pure thought.

NECESSITY FOR THEORY

While the first step in a structural analysis of ion accumulation is obviously the experimental analysis of structure, the second step—a theoretical analysis of structure—is perhaps not so obvious. Just as the analysis of electrical properties of complex tissues requires an explicit structural theory (8, 9, 16), so does the analysis of ion accumulation. A structural theory describing changes in ion concentrations is needed before measurements can be convincingly interpreted. To be sure, much experimental work can be done without *explicit* reference to a model or theory, but such work often uses an implicit model; experiments are interpreted as if the extracellular space were an "unstirred layer," a single compartment with uniform composition. It is obvious that an explicit model is preferable to an implicit model because it is more informative. It also seems clear that an explicit model is safer than an implicit one because explicit assumptions are easier to prove false than implicit ones.

Experimental work involving ion accumulation should be interpreted with an explicit, anatomically determined model, rather than with models that have convenient mathematical properties. Only in this way can components of the model correspond to the components of the tissue; only in this way can properties measured be interpreted as properties of individual biological structures.

THEORETICAL TECHNIQUES

It is, of course, easier to require such a theory than to produce one. The theoretical analysis required to predict the linear electrical properties of complex structures is not trivial (see the papers reviewed by Peskoff and Eisenberg (19), and Eisenberg and Mathias (9)), and the analysis necessary to deal with ion accumulation can be expected to be much more involved (e.g., Barry and Adrian (5)). It is important to see why this is so. The structural complexity and membrane properties of tissues more or less guarantee that the model describing such tissues will consist of partial differential equations with boundary conditions, specified on a boundary with complicated geometry. The analysis of electrical properties uses a familiar partial differential equation, namely Laplace's equation, along with a fairly difficult boundary condition. If one is concerned with only linear electrical properties, much of the difficulty of the mathematics can be removed by singular perturbation theory (19). The complexity in the mathematics associated with the complexity in membrane geometry has also been reduced to manageable proportions, using a combination of heuristic and rigorous techniques (8, 16).

In this manner, structural analysis of linear electrical properties has been made possible. But the linear electrical properties of tissues are rarely the properties most relevant to their function. Most often the function of tissues is produced by nonlinear properties, like active transport systems or ionic conductances. In that case, a structural analysis is more difficult, and it seems that no structural analysis of nonlinear properties has yet been done. But such an analysis is both necessary and possible. The techniques developed for the linear case can be used repeatedly, each time with the preparation at a different potential. That is to say, small perturbations in potantial can be applied to the preparation, starting from different "resting" potentials, and the resulting perturbation in current can be measured. Some of the circuit components identified in this manner will consist of the components that arise from electrolyte solutions and the lipid component of membranes—the same structural circuit elements identified in a strictly linear analysis. Additional circuit elements will appear, however, produce by the linearized properties of ionic conductances (13) and voltage-dependent capacitors. The measurement of these linearized nonlinearities—perhaps best called "ionic reactances"—will give considerable (perhaps complete) information concerning the distribution of nonlinear properties among the structural components of the tissue. In any case, the parameters of the "ionic reactances" will severely constrain the fit of a complete structural theory of the nonlinearities. (A complete theory is one that describes the response to all changes in potential, large or small.)

These techniques can also be applied to the problem of ion accumulation, but it is hard to guarantee their success, even in the linear case. The partial differential equations that describe ion flow are more complex, involving at least the concentration of each electrolyte, the rate of change of that concentration, the electrical potential, the activity of water, and the volume of the intra- and extracellular compartments, as described in Bockris and Reddy (6, see Ch. 4) and Barry and Adrian (5). In the nonlinear case the boundary conditions are fearsome, involving a logarithm and nonlinear ionic permeabilities—both of which resist linearization in many important situations (5). The utility of perturbation theory has not been established for these problems and so structural analysis of ionic concentration changes may well require a numerical, instead

of analytical, model. Models that must be evaluated numerically will complicate the task of structural analysis, since curve fitting to a numerical model involves considerable difficulties. Finally, the mathematical description of individual ionic permeabilities is a prerequisite for a structural analysis of nonlinear processes; one must know how the ionic permeabilities vary with voltage and concentration in order to perform a structural analysis.

These difficulties imply that structural analysis of ion accumulation will be awkward in the nonlinear case, particularly if one wishes results as precise as those obtained in the structural analysis of linear electrical properties. But the understanding of ion accumulation *requires* a structural analysis. So one must proceed with gritted teeth, trusting that difficulties will be resolved just as other forbidding difficulties were resolved in the structural analysis of electrical properties.

EXPERIMENTAL DATA AND ITS ANALYSIS

Once a theoretical analysis of ion concentration changes is available, experimental data can be used to determine the properties of structural components of the tissue, at least in principle. There are several kinds of experimental data available for this purpose. Our attention is restricted to electrical measurements, since they are almost always faster, more direct, or more precise than measurements of concentrations. The electrical response to changes in ion concentration in the bath can be measured (12), since the slow components of that response are often produced by changes in ion concentration. The electrical response to applied current or voltage can also be measured. Again, the slow components of the response are often produced by changes in ion concentration (2). Finally, natural activity, such as repetitive action potentials, can be used to change ionic concentration in particular compartments of the tissue (11).

The localization of the parameters that describe ion accumulation will almost always involve the fitting of a theoretical function that contains many parameters to experimental data taken in one of the situations just mentioned. The goal of the curve-fitting procedure is to determine as specifically as possible the best values of the parameters, which give the least deviation between theory and experimental results. It is hoped that these values are good estimates of the properties of individual structural elements.

The dangers of curve fitting are well known. Often the experimental data are not rich enough to determine all unknown parameters, and guesses or assumptions have to be made. Even when the number of parameters is manageable, it is difficult to know how well they are specified by the experimental data. One always fears that some quite different combination of parameter values might fit the data as well. Finally, data taken in the time domain—for example, the time course of a concentration—are notoriously recalcitrant to curve fitting, because the estimate of parameters is sensitive to small errors or noise in time domain data (Acton (1), p. 252, and Lanczos (15), p. 274). These problems imply that fitting experimental data with a theoretical model of ionic accumulation is unlikely to determine all the parameters of the model. Experimental data of ion accumulation is unlikely *in itself* to allow the measurement of the properties of components of tissues.

Fortunately, it is not necessary to determine the properties of *all* components of tissues from data on ion accumulation if a structural analysis of electrical properties has been performed on the same tissue. A structural analysis of electrical properties would determine the areas of membranes (from both morphometric and capacitance measurements) and the effective resistance of extracellular and intracellular spaces. These parameters appear, in only slightly modified form, in any model of ion accumulation and so they could be taken as constants when curve fitting to data on ion accumulation. Then curve fitting to transient data describing ion concentration changes would not need to determine the areas of membranes or the effective diffusion constant of extra- or intracellular spaces.

Since measurements of transients of concentration will rarely allow determination of all the parameters that appear in a structural model, a complete analysis of ionic concentration changes will *require* analysis of both electrical and diffusional properties. This has surely been the case in frog skeletal muscle. The electrical and diffusional properties of the T-system of frog skeletal muscle have been analyzed and it is clear from the work of Nakajima's laboratory (14, 17) that morphometric, electrical, and diffusional data—and structural theory—are needed to perform a complete analysis.

SUMMARY

Ion accumulation or depletion occurs in tissues with complex infolded membranes. Such tissues also have complex electrical properties, reflecting the complex circuit required to describe the flow of current. Many of the parameters that characterize ion accumulation or depletion also appear in the circuit model of the tissue. Therefore, the analysis of ion accumulation and the analysis of electrical properties should go hand in hand.

B. Eisenberg and R. T. Mathias have shared in the structural analysis of physiological properties, even before we called it that. It is a pleasure to acknowledge their continuing contributions and to thank them and R. Levis for reviewing this paper.

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