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Electrical Field Problems in Muscle and their Meaning to Mathematicians, Physiologists, and Muscle

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ABSTRACT: Muscles, like other biological cells, use electricity to perform their natural function. Contraction is coordinated by the action potential, a propagating solitary wave of voltage. Understanding the biological function of muscle thus requires the systematic analysis of the electric field in a complex structure. The analysis starts with measurement of the structure; it proceeds with a prediction of the electrical properties expected from that structure; it then compares the predictions with electrical measurements, particularly of impedance. The analysis concludes with an understanding of the biological function of the solitary wave.

Applied mathematics has essential roles in each step of the analysis. The quantitative analysis of structure uses stereological methods, an application of geometrical probability theory. The prediction of the electric field uses singular perturbation theory to solve electric field equations in a physically meaningful manner. The measurement of impedance involves statistical estimation of linear systems using stochastic signals. And understanding the propagation of the solitary wave depends on the theory of nonlinear wave equations. Analysis of electrical properties of biological tissues thus benefits from the clever use, even the invention of relevant mathematics.

INTRODUCTION

Muscle fibers contract--that is the function for which they have evolved and it is fitting that the papers in this symposium

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have focused mostly on the mechanisms of contraction. My interest lies in a different area of muscle biology, even biology in general, namely in the control systems which govern the contraction of muscle.

The mechanisms which control the mechanical output of contractile cells are of great importance, whether the control is on the short time scale of a twitch, the intermediate time scale of a contracture, or the long time scale on which a muscle fiber changes from one type to another: the control systems may be the key to a large fraction of the biologically and medically interesting properties of muscle. The constraints on the control mechanisms are weak compared to the constraints on the contractile system itself. The contractile system must produce mechanical output with biological efficiency, working within the laws of physics and chemistry, using the repertoire of structures and proteins encoded by the genome. On the other hand, the control mechanisms need not be chemically or mechanically efficient, because they consume a negligible fraction of the cell's free energy. One may therefore expect the control mechanisms to be more plastic than the contractile system itself; and so the control systems may contain the evolutionary adaptations most accessible to pathological corruption (i.e., disease) as well as experimental and clinical manipulation.

Control mechanisms in many systems (besides muscle) are as significant as the main effector itself. To pursue a metaphor apt for Detroit, where I present this paper, consider the mechanisms which control the engine in an automobile. These are at least as interesting as the mechanisms of combustion within the cylinders of an automobile engine! And, the control mechanisms are much easier to modify for our own ends than are the fundamental mechanisms involved in the production of power, since the mechanisms of power production are tightly constrained by the needs for power and efficiency, which do not apply so

strictly to the control mechanisms. That is presumably why microprocessors are used to perform the control function in internal combustion engines today.

Control mechanisms in muscle have a further interest, as we shall see. These mechanisms have much in common with many other mechanisms in biological cells. They are fundamentally electrical in nature and involve a subtle interplay of the structure of the tissue, of the properties of ionic channels which allow current to flow across membranes, and of the distribution of those channels through the structure. In this regard the control mechanisms of muscle illustrate the main themes in the control of solute movement and current flow across all cells. The nervous system communicates and performs its functions electrically, depending on the properties and distribution of channels and the structural complexity and organization of cells. Tissues specialized for the transport of solutes (such epithelia as kidney and intestine) depend on just the same mechanisms for their function. The properties and distribution of channels, and the structural complexity and organization of cells is just as important for the transport of water and salt in the kidney as it is for the integration of signals in the nervous system, or the control of contraction in muscle. Thus, the analysis we will speak of today, primarily in the context of muscle, is quite similar to the analysis appropriate for many other tissues.

COORDINATION OF CONTRACTION: EXCITATION-CONTRACTION COUPLING

Having put electrical problems in a broad context, let me now turn to specifics concerning the coordination and control of contraction in muscle. One of the important problems a muscle must solve is the coordination of its contraction. If a muscle is to develop useful work at its tendons, the contraction of its cross-bridges must be coordinated, both down the length and

across the width of the fiber. That is the main role of the action potential, which is a propagating solitary wave of voltage, which spreads longitudinally down the length of the fiber and radially across its cross section.

The action potential of skeletal muscle, like all electrical properties is determined in large measure by membrane properties. Membranes of cells are the main barriers preventing movement out of cells. Membranes serve the essential homeostatic function of keeping important substances close together. Indeed, membranes in their pristine state are so good at restricting movement that almost nothing of biological significance can cross them. Thus, membranes are punctuated by specialized protein molecules akin to enzymes, called channels, which allow the movement of important substances in and out of cells. Membranes and the channels in membranes control the flow of charged solutes, that is to say ions, and in this way they control current flow across membranes.

A number of membrane systems, containing a variety of specialized channels, are involved in the control of muscle contraction. The outer membrane of a muscle fiber supports the longitudinal propagation of the action potential, which ensures longitudinal simultaneity of contraction. The outer membrane of muscle fibers is invaginated into a network of branching tubules called the T system. The radial propagation of the action potential in the T system is responsible for the radial simultaneity of contraction. Within the muscle fiber is a separate compartment called the sarcoplasmic reticulum. The membranes of the sarcoplasmic reticulum are responsible for the release of calcium and its reaccumulation. That calcium in turn controls the contractile activity of the cell. The recent volume of the Handbook of Physiology (Peachey and Adrian, 1983) provides a series of review articles and references to the original literature in this field.

I will not discuss the action potential and its longitudinal propagation, both because this is classical material by now, and because a serious discussion of the mathematics of this subject would take the entire talk.

RADIAL PROPAGATION AND CURRENT SPREAD IN THE T SYSTEM

I will now turn to the lesser known questions of its radial spread in the complicated branching network we call the T system. The task of applied mathematics is to tell us the role of structure in this radial propagation.

There are several possible approaches to this problem. The classical approach in experimental physiology has been to ignore the branching network and treat the system as a disc of membrane, under the biologically plausible assumption that the microstructure is on too short a length scale to influence the macroscopic properties very much. This approach is more reasonable than it seems; indeed, the macroscopic properties are less dependent on the detailed structure than it might seem. But a price is paid if we restrict our gaze to the purely macroscopic --we cannot predict the main function of the T system, namely the radial propagation of the action potential, because that depends critically on the effective radial resistance of the T system, which in turn depends strongly on the details of the branching.

One way out of this problem is to simulate the entire T network on a computer as Mathias and I did some years ago (Mathias, 1975; Mathias, Eisenberg, and Valdiosera 1977). Equivalently, one can set up an analytical matrix of equations for the branching network and solve the matrix, at least for the small networks found in heart muscle (Levin and Fozzard 1981). We have felt that neither approach was very productive. Brute force simulation gave little insight, and in many cases was both harder and less useful than real experimentation. The matrix

approach gives expressions too complicated to understand, as soon as the networks have any realism.

An analytical approach to the problem is possible (Mathias et al, 1977) if one proceeds in the time honored tradition of assuming that which we cannot prove. Mathias and I developed a construction for the random branching network of the T system in which each node was classified to a shell, depending on its electrical distance from the outer surface of the fiber. If we assume that the potential at each node in a given shell is the same, we can set up difference equations which permit accurate approximation. As is so often the case, the resulting solutions can be exactly interpreted in terms of a simple circuit diagram, each element of which has a known and precise relation to morphological structures of the fiber. The use of circuit diagrams is sometimes a source of discomfort for applied mathematicians. But it is important to realize why they are so useful. First, circuit diagrams summarize the pathways for current flow, the electrical structure of the system (Eisenberg and Mathias, 1980). And most people find a pictorial representation easier to understand than that implicit in a differential equation or its solution. The circuit diagram also is an exact statement of the solution to a problem if it is the result of a proper mathematical analysis and not an inspired guess. Finally, the circuit diagram has a life of its own. The mathematical analysis is only possible under a restrictive set of conditions, for example with the assumption of linearity and isopotentiality. There is every reason to believe (indeed simulations show) that the essence of the analysis is valid under far wider and more relevant conditions. The circuit diagram implies the correct generalization for these wider conditions.

At this stage our discussion can turn in several directions. If we restrict our thought to skeletal muscle, we must now turn to the other membrane mechanisms of importance. That

is the biologically correct approach, but it is mathematically much less interesting since the role of applied mathematics is, up to now, fairly unimportant in this work. Or we can turn to other tissues and continue our analysis of T systems there. In other words, we can apply the insights developed to analyzing the extracellular space in other tissues.

I shall take that approach, returning in a rather surprising way, at least to us, to some major problems in skeletal muscle, at the end.

SYNCYTIAL TISSUES

We turn now to a class of tissues called syncytial that contain extracellular spaces entirely analagous to the T system (Eisenberg, Barcilon, and Mathias, 1979). Syncytial tissues consist of many cells electrically coupled together. The cytoplasm of these cells forms one medium through which current can flow. For example, current applied with a micro-electrode can flow through the cytoplasm, across the outer membrane, and into the outside world. The space between the cells of a syncytium forms another medium through which current can flow. This medium is entirely analagous to the T system of skeletal muscle, which we have studied so exhaustively.

Analysis of syncytial tissues requires first a derivation of the appropriate conservation laws. This again first requires a heuristic approach assuming the existence of a small cube of homogeneous tissue, somewhat analogous to the shells previously defined in the T system. With this assumption, differential equations can be derived to describe current flow in both the intracellular and extracellular media. The exact solution of these equations is possible, but essentially useless because the resulting expressions are too complex. The solutions, because they are general as well as exact, must describe many situations of little relevance and thus obscure the relevant properties in a plethora of terms.

Fortunately, we can apply the systematic approximation procedures of singular perturbation theory to remove this problem. Singular perturbation theory is particularly well suited to membrane problems because those problems are characterized by a biologically natural small parameter, namely the permeability of the membrane. As we have discussed, membranes exist to isolate the interior of cells from the outside. Thus, the permeability, expressed in suitable units, is always small. Using these techniques, we have derived simple approximations to the solutions of these equations and shown their applicability to two important syncytial preparation, the lens of the eye (summary and references can be found in Rae, Mathias, and Eisenberg, 1982) and sheep Purkinje strands (Levis, Mathias, and Eisenberg, 1983).

ELECTRODIFFUSION

So far in this paper I have only discussed the strictly electrical properties of these extracellular spaces. But all current flow in biology is by the movement of ions. Thus, current flow in biology is always accompanied by a change in the concentration of ions. This concentration change can be surprisingly important (Levis, Mathias, and Eisenberg, 1983). For example, if we write conservation laws for and then do numerical simulations of calcium movements in, say, Purkinje strands of the sheep, we see that dramatic effects of concentration change are expected on the shape of currents flowing during the action potential.

Up to now, almost all the work in this area has depended on simulations, with all the problems I alluded to before. The stage is now set for a serious analysis of electrodiffusion. We know it is an important process physiologically; we know the time scale and expected magnitude of the effects from simulations. Serious applied mathematics is now needed to give us

insight and useful approximations so we can deal with simultaneous current flow and diffusion as successfully as we have already dealt with current flow itself.

It is now time to fulfill my threat and return to the questions of skeletal muscle. What role does electrodiffusion have there? Electrodiffusion in the T system of skeletal muscle has been known to be of great importance in several experimental phenomena for a long time (e.g., see references and results in Eisenberg and Gage, 1969). But its relevance to functional questions has not been so clear. For example, what does electrodiffusion have to do with the central unanswered questions of excitation-contraction coupling, namely the question of T/SR (i.e., sarcoplasmic reticulum) coupling?

Recently, a great deal of attention has been paid to the mechanisms linking a voltage change across the T membrane to the release of calcium from the sarcoplasmic reticulum (Chandler, Rakowski, and Schneider, 1976; Schneider, 1981). One of the possible mechanisms involves the movement of calcium from the lumen of the tubules through a specialized messenger channel into the region between T membrane and sarcoplasmic reticulum and the recent discovery of the paralyzing action of a calcium channel blocker (Eisenberg, McCarthy, and Milton, 1983) supports the latter idea.

But the role of calcium entry in T/SR coupling has received little attention for many years because skeletal muscle fibers are known to contract in the absence of calcium <u>in the extracellular bathing medium</u>. This result has been taken to mean that calcium flux across the T membrane is not involved in excitation-contraction coupling. The reasoning was that calcium was absent in the T lumen, thus calcium could not flow across the T membrane; thus calcium flux could not be an essential step in excitation-contraction coupling.

It may turn out that this reasoning is incorrect, because

of the restricted nature of the extracellular space in skeletal muscle and the dominating effect of electrodiffusion in the T lumen. Consider, for example, the consequences of a vigorous calcium pump in the T membrane, particularly in the presence of a calcium binding protein in the T lumen. In that case, it might not be possible to deplete calcium in the T lumen simply by removing calcium from the extracellular bathing solution. The calcium binding protein might greatly impede the diffusion of calcium without interfering significantly with the diffusion of monovalent ions (as suggested to me by Stuart McLaughlin). The pump might be able to dominate the concentration of calcium in the T lumen. And finally the calcium binding protein might provide a reservoir able to support a few contractures of many twitches when the muscle fiber is bathed in calcium deficient solutions. The restrictions imposed by electrodiffusion in the T system may prove to dominate the experimental situation.

Of course, this speculation must be recognized as just that; it is useful because it suggests experimentation which can give definitive answers. The hypothesis motivating an experiment must never be confused with the result of the experiment! Nonetheless, this is a productive and provocative idea, which is currently motivating experiments aimed at evaluating the role of calcium flux in T/SR coupling. In this way, the analysis of electrodiffusion in syncytial tissues has motivated the study of an important mechanism in skeletal muscle, just as the analysis of current flow in the T system of skeletal muscle previously motivated the analysis of syncytial tissues.

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