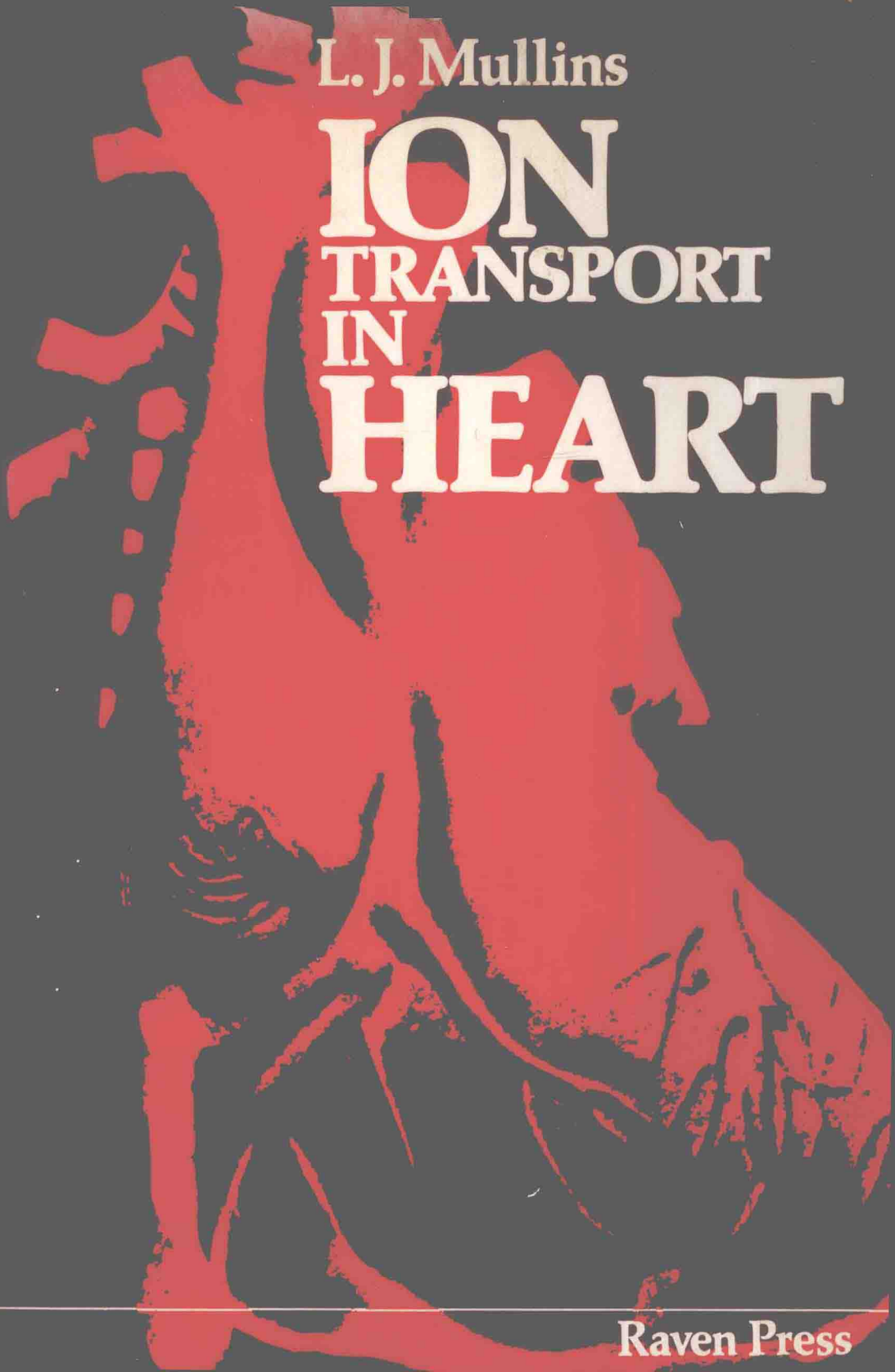
L. J. Mullins

ION TRANSPORT IN HEART



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Ion Transport in Heart

L. J. Mullins, Ph.D.
Professor of Biophysics and Chairman
Department of Biophysics
University of Maryland School of Medicine
Baltimore, Maryland

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ION TRANSPORT IN HEART

To Professor Silvio Weidmann
on the occasion of his 60th birthday
April 1981

Preface

In working out the details of Na/Ca exchange in squid axons, it seemed reasonable to ask: Can these principles be applied to other cells? More particularly, is there a cell with a very long duration action potential so that its electrochemical gradient for Na is greatly decreased for a significant period of time?

The answer to this question led directly to the development of this volume, because the reply was yes: the cardiac cell has all the properties one would expect if a significant control of contraction resided in a carrier-mediated rather than a channel-mediated process. Moreover, not only Ca entry but also its exit from the cell could be brought about by a single entity: Na/Ca exchange. This fact provides the rationale for the approach of this volume.

Because this book attempts to look at one particular phase of cardiac physiology—transport—the papers cited are those that I analyze in detail to illustrate a particular point. My purpose is not to review the field critically or to deny the existence of other, contrary views. It is general experience that the membrane-bound entities we call the Na/K pump, the Na channel, or Na/Ca exchange are universally distributed. Hence, this volume suggests that the long duration of the cardiac action potential has been developed for one and only one purpose: to reduce the Na electrochemical gradient for an appreciable period of time.

The treatment of the subject is largely nonmathematical and has been designed for students and research workers with a knowledge of cardiac electrophysiology, but without experience in ion transport across membranes. Chapters 1 through 4 provide some background in transport useful for these readers. Coverage of all chapters overlaps so that each chapter can be read and used individually.

L. J. Mullins
April 1981

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Contents

Chapter 1

Introduction

Studies of cardiac electrophysiology have been concerned with an identification of the currents that flow during a voltage clamp. These follow the classic studies of Hodgkin and Huxley, who used an analysis of voltage clamp currents to specify the patterns of conductance changed to both Na and K that underlie the generation of an action potential in nerve. In cardiac muscle, however, the recognition that there is a slow inward channel (Ca current) in addition to Na and K channels and the suggestion that K movement is fractionated among a variety of different types of K channels have led to great difficulty in analyzing voltage clamp data in classic Hodgkin-Huxley terms.

It has long been recognized that a Na pump is necessary to reverse the dissipative movement of Na involved in the generation of an action potential in nerve. If one could add to this requirement that of a Ca pump to reverse the dissipative movements of Ca, it might be possible to explore cardiac electrogenesis not by measuring the highly complex dissipative currents, but by analyzing the somewhat simpler restorative currents. The movement of K is largely ignored, since a combination of anomalous rectification and a membrane potential close to E_K (except in nodes) combine to make this sort of dissipative ion movement small compared with those of Na and Ca.

Much of the information about restorative ion movements (i.e., transport) comes from recognizing that two separate mechanisms are capable of producing a Na efflux. The first is the conventional Na/K pump; the second is the Na/Ca transport mechanism. Since the latter mechanism is usually thought of as pumping out Ca in

exchange for the entry of Na, it is helpful to describe the Na efflux produced by this transport as a "running backward." While the Na/K pump has been caused to "reverse" and produce ATP while dissipating Na and K gradients, the requirements for this mode of operation are not likely to be met under physiological conditions. Thus Na/K exchange may be viewed as always running forward, while changes in membrane potential and $[Ca]_i$ and $[Na]_i$ are capable of reversing Na/Ca exchange.

The purpose of this volume is to emphasize the role of Na/Ca exchange in accomplishing the following during each heartbeat: (a) stopping the outward transport of Ca and initiating an inward Ca transport during the plateau, and (b) reversing the direction of carrier-mediated Ca transport during the diastolic interval so that not only the Ca gain from (a) above but also the Ca gain from I_{Ca} moving through slow inward channels is reversed. A role for Na/Ca exchange is present in the initiation of both contraction and relaxation. For Na/Ca exchange to behave in this way, the system must be electrogenic (i.e., transport more than two Na per Ca); the evidence for such an electrogenic mode of operation is listed below and is discussed in detail in subsequent chapters:

1. A $[Ca]_i$ of 20 to 50 nM that is appropriate to a relaxed muscle fiber can be produced only by a coupling ratio of four Na to one Ca (1).
2. Ca efflux in squid axons (2) varies as $\exp(-E_m F/RT)$, with F representing Faraday, R a gas constant, and T temperature. Ca influx that is coupled to Na exit varies as $\exp(E_m F/RT)$. This is appropriate voltage sensitivity of a carrier moving two net charges per cycle.
3. Measurements analogous to the preceding point made by depolarizing (3) cardiac fibers with KCl are not satisfactory. Such treatment changes the ^{45}Ca specific activity by the entry of unlabeled Ca and also makes changes in $[Ca]_i$ by the release of Ca from the sarcoplasmic reticulum (SR).
4. Small changes in $[Na]_i$, such as those produced by clinical doses of ouabain, yield large changes in Ca entry (4). Since at constant E_m the entry of Ca is given by $[Ca]_0([Na]_i)^r$, where

r is the coupling ratio Na/Ca, a value for r in excess of 2 is indicated by these measurements (1).

5. Tension continues to increase in cardiac fibers clamped at or beyond E_{Ca}. The evidence is convincing that the Ca producing contraction comes from outside the fiber (5); only a nonelectroneutral Na/Ca exchange can explain this effect.
6. Membrane vesicles isolated from cardiac myocardium and studied *in vitro* show an electrogenic Na/Ca exchange (6).

An important role for Na/Ca exchange in influencing contraction was suggested by the pioneering work of Repke (7) and at the same time by Langer (8); this was 4 years before the classic paper of Reuter and Seitz (9) that suggested a Na/Ca countertransport. Problems exist in accepting the idea that Na/Ca exchange might move Ca both into and out of cardiac fiber. First, it is difficult to make the isotopic flux measurements necessary to prove the hypothesis; these require squid giant axons. Second, skeletal muscle physiology, with its dominant SR Ca release, has unduly influenced thinking about cardiac fibers.

A further difficulty in initiating transport studies may have been that voltage clamp methods were applied to cardiac fibers long before an appropriate analysis of the errors that might be involved. Thus a substantial literature concerning artifacts was built up and only recently have there been realistic efforts to analyze ionic currents in cardiac fibers.

There is at present great interest in the extent to which Na/K pumping can affect the parameters of cardiac contractility; only a short while ago electrical measurements and ion transport studies were separate fields, with little interconnection. It is now known that not only Na/K transport with its contribution to membrane potential and to $[Na]_i$ but also Na/Ca exchange may be intimately involved with excitation-contraction coupling. This presages a new era of research that will address not only dissipative ion movements but also restorative processes.

The quantitative aspects of Na/Ca and Na/K exchanges cannot be discussed without assumptions about actual values for $[Na]_i$ and $[Ca]_i$. First, we assume that $[Na]_i$ is 10 mM in resting, that is,

noncontracting, myocardium, and may be 20 mM when the heart is beating and hence subject to a substantial Na load. These assumptions do not differ from Na electrode measurements of an activity for Na^+ of 7 mM and an activity coefficient of 0.75, which would make $[Na]_i = 9.3$ mM in resting fibers. Substantial reservations about ion-specific electrode measurements are discussed in this volume. The rarefied thermodynamic concept of ion activity in cardiac fibers is perhaps best dismissed considering the rather primitive state of the art of its measurement. Recently, electrode measurements of $[Ca]_i$ have begun to appear. The greater difficulties of an experimental sort that exist here suggest the need for caution about embracing a particular value. Among these difficulties are the following: (a) calibration is with CaEGTA/EGTA buffer, yet realistic calibrating solutions are seldom used; the myoplasm has a high ionic strength since the principal anions are ATP^{4-} and CrP^{3-} and the dissociation of CaEGTA is sensitive to ionic strength; (b) the penetration of a myocardial fiber with a large electrode almost surely disrupts much SR with a consequent change in Ca buffering; (c) the leak induced by a large electrode in a myocardial cell must be expected to depolarize it and hence to lead to a larger steady state $[Ca]_i$ than normal; it also leads to a Na leak that is quantitatively much larger than any Ca leak, since Na_o/Ca_o is of the order of 75. Such a Na leak must be expected to perturb $[Ca]_i$.

The values used are for $[Ca]_i$ determined in the squid giant axon where none of these problems with electrodes can arise, and where the dissociation constant of CaEGTA has been determined at the precise ionic strength existing in axoplasm. This involves values of 30 nM for the $[Ca]_i$ in resting fibers and higher values as $[Na]_i$ is increased. None of the arguments about Na/Ca exchange critically depends on exact values of $[Na]_i$ and $[Ca]_i$ but only the potential where reversal of Na/Ca exchange takes place.

The following notation is used: [ ] denotes free ion concentration, where the subscripts i and o refer to inside and outside the membrane, respectively; but Na_i and Na_o, for example, are employed to represent internal and external ionic species where no reference to a concentration is intended. Currents I_{Na} and I_{Ca} are the actual

net fluxes of the particular ions, whereas I_{si} is the mixed current through a particular channel. A new current, I_C, is introduced for the carrier current of Na/Ca exchange. Note that in the balance of the text the ionic charges belonging to the different species are dropped but should be understood.

The presentation is organized first by a consideration of carrier-mediated transport in general, followed by a treatment of Na/K transport, Na/Ca transport, and the interactions among these systems. Finally, use is made of these considerations of ion transport to explain how the cardiac action potential is able to control the production of both tension and relaxation.

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Chapter 2

Carrier-Mediated Transport

DIFFUSION VERSUS CARRIERS

Channels

The large currents measured across the excitable membrane by electrophysiologists have required the development of a structure that could accomodate such ion traffic. Phospholipid bilayers are not a promising medium for ion movement since their specific resistivity is high; the incorporation into a bilayer of a protein whose core is predominantly aqueous while its periphery remained lipid-like makes possible the channel concept. Channels can be gated, meaning that stimuli can open or close the channel to ion traffic, or they can be continuously open structures. Diffusion in the structures is approximately that given by Fick's law.

Carriers

An older concept in membrane studies is that of a carrier; it is an organic molecule that is highly lipid soluble and hence remains in the membrane phase. Again, the idea is to provide a way around the diffusion barrier imposed by the phospholipid bilayer. Originally, carriers were ferries that crossed back and forth carrying substances from one side of the membrane to the other. With the isolation proteins with sizes of 250,000 daltons, this arrangement is less plausible. With the further requirement that carriers have binding sites on both sides of the membrane that must be filled at the same time, there is more of an inclination to regard carriers as

continuous from one side of the membrane to the other. The difference between channels and carriers may be only that we assign relatively slow movements and those with high specificity to carriers, while structures allowing more rapid and less specific solute movements are called channels. Diffusion by carrier is easily saturable, a property not associated with channels.

FACILITATED DIFFUSION

The movement of substances across the cell membrane is via the intervention of a carrier or substance that is confined to the membrane phase and combines with the carried species of ion or molecule at one membrane surface and releases the species at the other membrane surface. For the reactions shown below, which are those involved in transport, X is the carrier; S is the substrate to be carried; as before, the subscript i stands for inside; and o, outside.

$$S_o + X_o \rightleftarrows SX_o \qquad [1]$$

$$SX_o \rightleftarrows SX_i \qquad [2]$$

$$SX_i \rightleftarrows S_i + X_i \qquad [3]$$

$$X_i \rightleftarrows X_o \qquad [4]$$

Reaction 1 is the formation of the diffusible carrier complex; 2 is the translocation of the complex, 3 the dissociation of the complex, and 4 the return of the unloaded carrier. If reaction 4 is made impossible, the reaction is called exchange diffusion since the fluxes of S in both directions are required to be equal. Reaction 1 can be defined by

$$K = \frac{([S]_o)\,([X]_o)}{[SX]_o}.$$

Since it is usually considered that the translocation reaction (2) is the rate-limiting step, it is the concentration of SX_o that will determine the flux, or

$$\text{Flux} = \frac{([S]_o)\,([X]_o)}{K}.$$

For low concentrations of S_o, $[X]_o$ remains approximately constant, so that flux increases linearly with $[S]_o$; for large values of $[S_o]$, saturation of the system will occur. The usual arrangement is: $[X]_o + [SX]_o = 1$; thus $K/[S]_o = (1-[SX]_o)$, where the term on the left is a reduced concentration. When $K = [S]_o$, then $[SX]_o = 0.5$, or the flux is half-saturated. Note that this mechanism will catalyze the dissipation of the gradient in S across the membrane, and a net flux of S will cease when $[S]_o = [S]_i$.

COTRANSPORT AND COUNTERTRANSPORT

In sharp contrast to the Na pump with its requirement for a substrate, ATP, there also exist in a variety of cells transport mechanisms that move sugars, amino acids, ions, and other substances in an uphill direction using as an energy source the Na electrochemical gradient. A single, ATP-consuming transport system, the Na/K pump, establishes the Na electrochemical gradient. Other modes of transport then draw on this secondary source of free energy for their requirements.

Systems are known in which Na^+, a sugar (S), and a carrier (X) combine in a ternary complex [Na-X-S]. This diffuses across the membrane and dissociates. The result is an increase in $[Na]_i$, which is rapidly reversed by the Na pump, and an increase in the concentration of S_i. This is cotransport and is a common feature of secretory epithelia, where substrates must be conserved. In some cases, the driving ion (Na^+) and the substance or ion to be transported are on opposite sides of the membrane. Thus when Na^+ enters, an ion from inside the cell is moved outward. In this case, the process is called countertransport. An example might be the efflux of Mg coupled to the entry of Na (1).

How much energy is available in the Na electrochemical gradient? The chemical part of the gradient is $\mu_C = -RT \ln([Na]_o/[Na]_i)$, while the fact that there are energetic consequences of the