Cellular Pacemakers

VOLUME 1
MECHANISMS OF PACEMAKER GENERATION

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Preface

This book is a result of an increasing awareness that many cells with seemingly very different functions use similar mechanisms and a desire to focus this awareness on the particular case of electrical pacemaker discharge. Endogenous pacemaker activity is a characteristic of a great variety of cell types ranging from the smooth muscle cells of the gut to the neurons of the highest parts of the brain. In different tissues these discharges trigger responses ranging from muscular contraction to release of neurotransmitters or hormones. Because of the role of endogenous electrical discharge in a variety of different tissues, an understanding of the mechanisms underlying pacemaker activity is essential in order to elucidate the normal and pathologic functions of the heart, brain, peripheral nervous system, gastrointestinal tract, and endocrine system of mammals. In all animals, at least some forms of behavior can be shown to originate as a result of pacemaker discharge. We are increasingly aware of the diseases of man that originate in cellular pacemaker malfunction.

The study of pacemaker discharge is important to biophysicists, pharmacologists, and physiologists specializing in the study of all of these organ systems, as well as to behaviorists and clinicians. This volume consists of contributions that deal with the biophysical analysis of pacemaker discharge in various cell types. In Volume II the emphasis is on the functional importance of pacemaker discharge and its role in human behavior and disease. These books are prepared to demonstrate that nature utilizes a common mechanism to achieve very different effects in a variety of cell types and to stimulate further consideration of roles for endogenous pacemaker activity in normal and abnormal cellular function.

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INTRODUCTION

PACEMAKER HAS BEEN DEFINED as "one who makes or sets the pace for another" or "a body part . . . that serves to establish and maintain a rhythmic activity." In this volume we are concerned with the generation of electrical signals in single cells. The term *pacemaker* will often be used to denote a single cell that can generate an action potential, the electrical signal that is fundamental to communication between neurons and to the generation of muscle contraction in the absence of any other outside influence. In many instances, such as the classic cardiac pacemaker, the action potential generated in a single cell does indeed set the pace for other cells and even for the whole organ. In other cases, however, endogenously active cells, such as neurosecretory neurons, may "pace" an entire behavior even when there is no direct cell-to-cell transmission of electrical activity. For our purposes, therefore, the term *pacemaker* will denote a cell that generates endogenous electrical activity without particular consideration of how that activity influences other cells.

It was well known in the seventeenth and eighteenth centuries that the heart of some animals would continue to show periodic contractions when removed from the animal and maintained in an appropriate solution. It later became clear that while some hearts depended upon a driving influence from nervous tissue (neurogenic hearts), others, like those of mammals, had inherent pacemakers (really a series of pacemakers) that were modified muscle cells. In retrospect, the existence of a rhythmic driving force from nervous tissue to heart in the neurogenic animals should have suggested a pacemaker role for neurons, yet it was not until the studies of Alving (1968) that neurons were shown indisputably to be capable of spike generation in the absence of synaptic drive from other neurons. In her experiments, Alving found that ligation of monopolar cell bodies with a fine thread abolished all action potentials generated by synaptic inputs. This observation was consistent with the known fact that in this preparation all synapses occur in the neuropile, not on the cell body. However, regular pacemaker discharges could still be recorded from the cell body in many neurons. Indeed, some neurons, which before ligation had a moderately irregular discharge as a result of an admixture of pacemaker activity and synaptic inputs, became perfectly regular when the synaptic interaction was terminated. Alving concluded that these neurons were endogenous pacemakers, although even in

pacemaker neurons action potentials could also be generated through synaptic mechanisms.

Spontaneous rhythmic contractions of smooth muscle have also been observed for a long time (Alverez & Mahoney, 1921), but identification of the site of generation has been complicated by the extensive neuronal networks in many tissues such as the gut. The electrical excitability of many glandular tissues is a relatively recent finding (Dean & Matthews, 1968). Even some nonexcitable tissues, such as fibroblasts (Nelson et al., 1972) and macrophages (Gallin et al., 1975), exhibit both spontaneous and elicited oscillations of membrane potential. While these are not action potentials, unlike the other rhythms to be presented, they are oscillatory and may have mechanisms of generation in common with other pacemaker potentials.

A major question concerning the mechanisms underlying the rhythmic events, whether observed at a cellular or organismic level, is whether or not the ultimate pacemaker generator can be understood solely in terms of an electrical, rather than biochemical, basis. The assumption of an electrical rather than a biochemical basis of rhythmic generation is implied even in our modification of the definition of *pacemaker*. There are many reasons for believing that rhythms are generated by electrical mechanisms. No biochemical mechanism has been found to be causative in any of the pacemaker action potentials in nerve cells, in smooth, cardiac, or skeletal muscle cells, or in gland cells. Furthermore, the known rhythms of circulating catecholamines, various tissue enzymes, and other biochemical parameters in mammals have all been found to depend upon electrical activity in a few parts of the nervous system, such as the suprachiasmatic nucleus (Brownstein & Zatz, 1982) or pineal (Binkley, 1982). However, electrophysiology is a science that describes events in terms of potentials, permeabilities, and currents and often does not explain the forces that generate these electrical signals. Thus a biochemical basis or at least influence for pacemaker discharge cannot be ruled out.

There are several known biochemical oscillating systems (see reviews by Hess & Boiteux, 1971, and Goldbeter & Caplan, 1976). One of the best understood is the oscillations of glycolysis, first observed in yeast cells but later seen in various mammalian cells and in cell-free extracts. These oscillations are due to the activity of a single enzyme, phosphofructokinase. Models of such a system stress the importance of negative feedback by product or substrate inhibition (Spangler & Snell, 1967) and, in the specific case of phosphofructokinase, the enzyme's autocatalytic and allosteric properties (Boiteux et al., 1975). The oscillations are observed by recording the fluorescence of NADH and are triggered by adding glucose. Their period is very temperature-dependent and is usually in the range of several minutes.

Several attempts have been made to relate electrical pacemaker discharge to oscillations of the glycolytic pathway. Chaplain (1979) reported that effectors that promote phosphofructokinase activity in the nervous system of *Aplysia* will stimulate pacemaker activity. However, this result is not particularly surprising, since the ability to generate pacemaker potentials de-

pends upon maintenance of a high membrane resistance (Carpenter, 1973), which in turn depends upon metabolism (Carpenter et al., 1971). Also it has not been possible to detect glycolytic oscillations with periods even approaching those for the rapid pacemaker discharge. Matthews (1975) has also proposed models of how glycolytic oscillations might be a factor in the generation of oscillations of membrane permeability in pancreatic islet cells (see also Matthews, Chapter 11, this volume), although no clear proof of his hypothesis is available.

Another important and interesting oscillation is the synthesis of cyclic AMP in cellular slime molds such as *Dictyostelium discoideum*. These cells have two stages, as independent amoebae and as multicellular fruiting bodies. Single cells aggregate in response to cyclic AMP, which is generated and released periodically and which then acts at membrane receptors of other cells. Cyclic GMP levels also oscillate and have a slight phase advance over the cyclic AMP oscillations. Calcium ion also alters the response (Gerisch et al., 1979). This system, with regulation of membrane proteins through activation of membrane receptors, appears to be particularly relevant to some of the mechanisms in a variety of pacemaker tissues where neurotransmitters modulate pacemaker discharge (see Chapters 4 and 8).

The slime mold *Physerum polycephalum* shows rhythmic contractions of its plasmodia. These contractions are dependent on cytoplasmic actomyosin (Wohlforth-Botterman, 1977, 1979). The contractions are associated with cyclic alterations in the intracellular concentration of free Ca^{2+}, and contraction occurs only during the phase of higher Ca^{2+} concentration (Ridgeway & Durham, 1976; Braatz, 1975). It is particularly interesting that this system also has slow oscillations in membrane potential, which are also in phase with the contractions. These potential changes presumably reflect oscillatory alterations in membrane permeability. Even more surprising is the existence of a fast but graded electrical response to mechanical stimuli. This response is accompanied by a marked increase in intracellular Ca^{2+} concentration and movement of the plasmodia (Ridgeway & Durham, 1976), a sequence very similar to those underlying many aspects of nerve and muscle physiology.

Mitochondria undergo oscillations in volume which are related to ion transport systems (Gooch & Packer, 1974). Several manipulations which stimulate ion transport induce this cyclic swelling. The swelling is associated with an increased transport, while the shrinking phase is associated with a decrease in metabolic and pump activity. It is the inner, not the outer, membrane which is responsible for the oscillation that may be related to oscillatory ATP levels.

Several other enzyme systems show oscillations under some circumstances (e.g., Goldbeter & Caplan, 1976; Israel et al., 1979). However, the significance of these biochemical oscillations remains to be determined. Many or all may be only indicators of negative feedback and may have no relation to those electrical events which are a common final mechanism in cellular pacemakers. Some, however, are clearly associated with an altera-

tion in the behavior as well as the biochemistry of component cells, as is the case of the production of cyclic AMP in slime mold.

Although the subject of biochemical oscillations is interesting and deserves close attention, the papers included in this volume will show that at least the regular beating pacemaker discharge of nerve, muscle, and gland cells can be explained solely on the basis of the electrical properties of the membrane. Like the generation of ordinary action potentials, the pacemaker spikes do not have short-term energy requirements as long as ionic concentration gradients and membrane resistance are maintained. They are generated by particular combinations of potential and resistance in membranes that have the appropriate time- and voltage-dependent channels.

These facts do not, however, mean that metabolic and biochemical oscillations are unimportant or without influence. Membrane potential and resistance both ultimately depend upon metabolism. Both also depend upon such factors as the concentration of intracellular Ca^{2+} (Meech & Standen, 1975), which is in turn dependent upon a mixture of permeability and transport processes and is interrelated with the cellular levels of cyclic nucleotides (Rasmussen, 1970). These interactions were reviewed in 1979 by Berridge & Rapp, who sought a biochemical basis for all oscillations. Although evidence for such a basis is not available, various metabolic processes can clearly alter the manifestation of pacemaker discharge. In some systems, such as plant rhythms (Bünning, 1982), it is not apparent what role electrical activity has in generating the rhythm.

While biochemical mechanisms are not necessary to invoke the generation of simple pacemaker action, there must be a biochemical basis for an altered expression of pacemaker discharge over time, particularly in the expression of rhythms with relatively long (e.g., circadian) periods. Unfortunately, this is an area where little information is available. The chapters in this volume will document the considerable information available on the ionic mechanisms underlying pacemaker discharge, the similarities between mechanisms in quite different types of cells, and the involvement of cellular pacemakers in a great variety of animal and human behaviors and diseases. There remain, however, many unanswered questions, particularly concerning the ways in which patterned and long-term rhythms are generated.

In spite of these unanswered questions, the information summarized in the present volume allows three important conclusions: (1) Endogenous electrical cellular pacemaker activity is a widespread phenomenon in the plant and animal kingdoms. (2) The mechanisms of pacemaker generation are similar, if not identical, in a great variety of cell types. (3) Endogenous pacemakers serve a wide variety of critical functions in different organisms.

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PART ONE

Cardiac Pacemakers