Advances in General and Cellular Pharmacology

VOLUME 1

Edited by T. Narahashi and C. P. Bianchi

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Preface

Knowledge of the mechanism of action of drugs at cellular, subcellular, or molecular levels is of vital importance not only in giving the basis of interpretation of the systemic action of drugs but also in improving existing drugs; in designing new forms of drugs; and in giving the basis of therapeutic applications. Classical pharmacology, concerning the action of drugs at integrated levels, does not necessarily give sufficient information as to the mechanism of action of drugs. A variety of sophisticated concepts utilizing the methods of physics, chemistry, biophysics, biochemistry, and physiology must be synthesized to understand the mechanism of action. Only since the last decade, however, have these techniques been fully applied to pharmacological investigations. It is of utmost importance to realize that a new dimension of pharmacological research has indeed emerged as a result of such a multidisciplinary approach; this approach is encompassed in general and cellular pharmacology.

Such recent studies of drug actions have led to a number of important findings. Certain chemicals and drugs were found to possess highly specific actions on cellular functions, so that they are widely being used as powerful tools for the study of a variety of physiological and pharmacological problems. Our knowledge of the cellular mechanisms of drug action has provided the basis for interpreting the systemic effects of the drugs and insight into the molecular mechanism involved.

For example, the puffer fish poison tetrodotoxin was shown to cause a highly specific inhibition of the sodium conductance increase in nerve membranes, thereby blocking impulse conduction. This stimulated research along this line and led to important contributions as to the mechanism of action of some other neuroactive toxins or agents such as batrachotoxin and local anesthetics. Applications of intracellular microelectrode techniques

to cardiac pharmacology have unveiled a variety of important features concerning the mode of action of antiarrhythmic and other cardiac drugs. Recent developments and applications of biochemical and physiological techniques have made it possible to clarify the nature of cholinergic and adrenergic receptors in the postsynaptic membranes, a contribution with an immense pharmacological impact and importance.

General and cellular pharmacology is a multidisciplinary field requiring integration of many fields of knowledge at a high level to achieve an appropriate understanding of drug action. It has developed rather recently and will continue to be a fertile field where a number of challenging accomplishments are expected to be made in the coming years. General and cellular pharmacology represents a modern-day approach to the mechanism of cellular drug action and provides a virgin field for many stimulating achievements in the future.

This new series of books, *Advances in General and Cellular Pharmacology*, is intended to provide a forum for this field. Each expert is to contribute an in-depth review on his own field from his own point of view. Therefore, one should not expect comprehensive review articles from this series of books, but to find newer approaches and techniques with solid theoretical and experimental bases.

Toshio Narahashi
C. Paul Bianchi

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Cardiac Cellular Pharmacology: Automaticity in Cardiac Muscle— Its Alteration by Physical and Chemical Influences

FRANCIS M. WELD AND
J. THOMAS BIGGER, JR.

I. AUTOMATICITY IN HEART MUSCLE

The heart rhythmically and spontaneously activates itself many times in a minute. The process responsible for this behavior has been termed the "normal automatic mechanism" and is a property of only a few cell types in the heart. Cells in the sinoatrial node, atrioventricular rings, and ventricular specialized conducting tissues possess the capacity for automaticity of this type whereas ordinary atrial and ventricular muscle cells do not. This mechanism not only is the basis for normal cardiac rhythmicity but also can generate arrhythmias in the heart. Although the normal automatic mechanism is complex and probably varies somewhat in different types of

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automatic cells and in different species, much is now known about the cell membrane behavior which underlies automaticity and about alterations in this behavior caused by a variety of physical and chemical influences. In this chapter, it is our intention to discuss the factors which generate and modify the automaticity brought about in heart muscle by spontaneous diastolic depolarization.

Early observations on the excitatory process in frog and tortoise heart were reviewed and extended by Burdon-Sanderson and Page (1880). Using external electrodes in a capillary electrometer system, they confirmed the inseparability of ventricular contraction and electrical activity and noted that cardiac rate was dependent upon temperature. With the first photographs of cardiac electrical activity (Burdon-Sanderson and Page, 1884), spontaneous atrial depolarization was delineated, as were induced atrial and ventricular depolarization, atrioventricular conduction delay, and intraventricular conduction delay.

Meek and Eyster (1914), without the benefit of intracellular microelectrodes, described the general behavior of the different cardiac pacemaker tissues in a way which has scarcely been improved upon to the present time. They delineated the progressively slower inherent firing rates possessed by the specialized cardiac pacemaker tissues "from above downward." This hierarchy of automaticity progressing from sinoatrial node to ventricular tissues results in control of cardiac rhythm by the "highest" center (i.e., the sinoatrial node) unless its inherent rate is depressed by influences such as the vagus nerve. Their concept of the hierarchy of pacemakers is still valuable in the analysis of pacemaker activity today.

Prior to microelectrode studies, arguments arose as to the nature of spontaneous rhythmicity—some authors favoring slow depolarization in pacemakers (Arvanitaki, 1938; Rijlant, 1936), others, oscillatory behavior (Bozler, 1943). The introduction of glass capillary microelectrodes allowed measurement of transmembrane voltage in single muscle cells (Ling and Gerard, 1949). The first demonstration of the time–voltage course of a cardiac pacemaker cell was by Draper and Weidmann (1951) in Purkinje fibers from sheep and dogs and by Trautwein and Zink (1952) in cells of the frog sinus venosus. These studies showed that automatic cells undergo spontaneous membrane depolarization during phase 4. In true pacemaker cells this process brings transmembrane voltage to the critical firing threshold to initiate a regenerative action potential. In potential pacemaker cells, such as those in the peripheral Purkinje system, spontaneous diastolic (phase 4) depolarization may or may not reach the critical firing threshold under normal circumstances. In still other cardiac cell types, such as working atrial and ventricular muscle, no spontaneous membrane depolarization occurs following repolarization of the action potential, since these cells totally lack

the membrane conductance properties capable of generating normal automatic behavior.

In the classical electrophysiological sense, automaticity resulting from spontaneous phase 4 depolarization depends on the four variables illustrated by the diagrams in Figure 1, where the transmembrane voltage–time course of a single cardiac Purkinje cell is shown. In each panel of Figure 1, a normal spontaneous action potential is shown as a solid line; the effects of modifying one of the four classical electrophysiological determinants of automaticity is shown in dashed lines. Figure 1A shows that a more rapid rate of phase 4 depolarization results in a more rapid attainment of the critical firing threshold ($V_{\text{threshold}}$) and thus a more rapid rate of spontaneous discharge. Slowing phase 4 depolarization has the opposite effect. Figure 1B shows that if the transmembrane voltage change during phase 4 depolarization is small, transmembrane voltage can fail to reach the threshold voltage, and thus abolish automaticity. Figure 1C reveals that a change to a more positive threshold voltage will result in a slowing of firing rate, while the converse is true for a shift to a more negative threshold voltage. Figure 1D reflects the different intervals between successive action potentials resulting from changes in maximum diastolic voltage: increases in maximum diastolic voltage slow the spontaneous firing rate; decreases in maximum diastolic voltage enhance spontaneous firing. Although these classical features of automaticity are conceptually simple, they are themselves determined by complex interactions between both passive and active membrane behavior.

Hoffman and Cranefield (1960) outlined the possible ionic causes for spontaneous phase 4 depolarization: (1) The resting membrane could show a gradual loss of potassium conductance. This would cause transmembrane voltage to move in a positive direction, toward zero, since the potassium equilibrium potential (see equation 7) is about -100 mV for usual intra- and extracellular potassium concentrations (140 mEq/liter and 3.5 mEq/liter, respectively). (2) A gradual increase in membrane conductance to sodium ions during diastole could equally well explain the spontaneous phase 4 depolarization, since the sodium equilibrium potential is about $+30$ mV (inside of the cell positive). (3) A decrease of electrogenic sodium pumping in the course of diastole would also result in spontaneous phase 4 depolarization, owing to a time dependent decrease in outward (repolarizing) current. (4) Finally, there might be other membrane ionic conductance changes (e.g., calcium) responsible for spontaneous phase 4 depolarization. There is now reasonable evidence that all four of these proposed mechanisms play a role in spontaneous phase 4 depolarization in one or more cardiac cell types. We will attempt to show in subsequent sections that the individual mechanisms contribute variably to pacemaker currents in different cell types, and—perhaps equally important—in the same cell type under different circumstances.

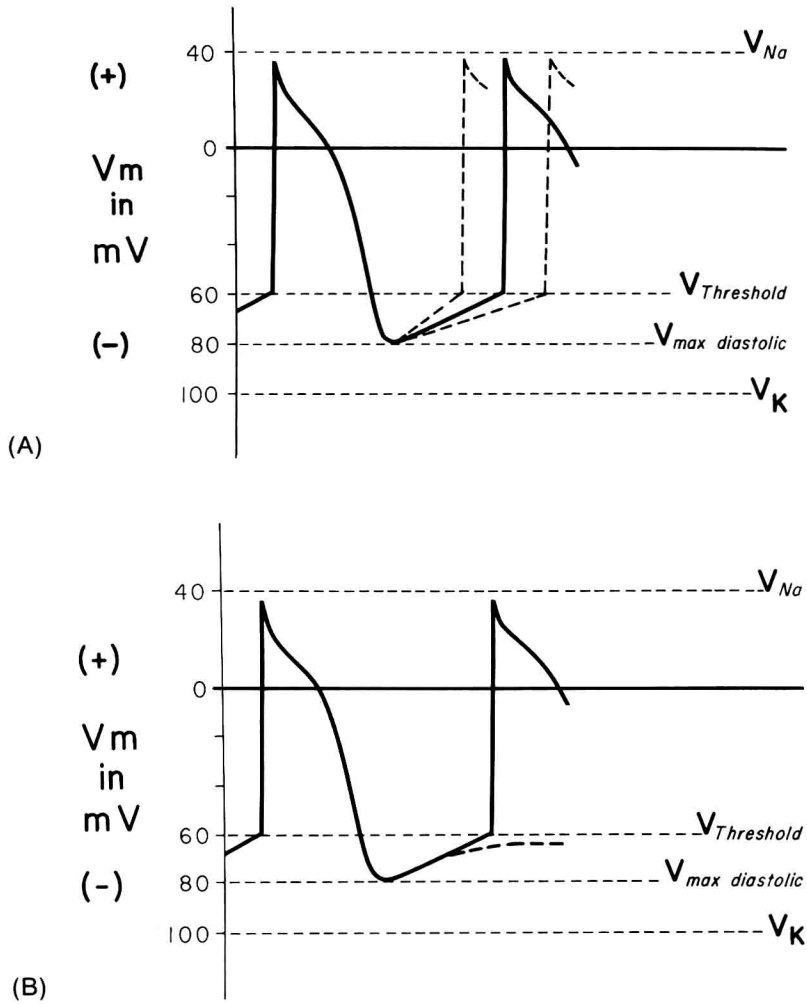
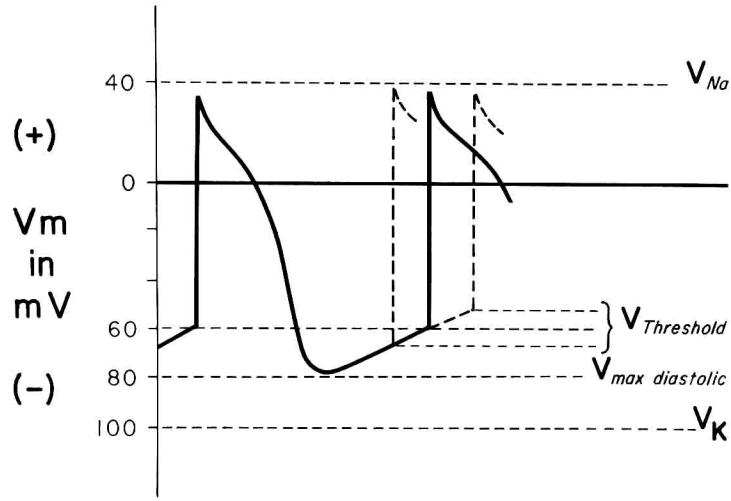


Figure 1. Electrophysiological determinants of automaticity. The solid lines of each panel represent the action potentials of an automatic fiber. The dashed lines represent changes caused by alterations in the following determinants of automaticity. A: Rate of phase 4 depolarization. B: Magnitude of phase 4 depolarization. C: Threshold voltage (critical firing threshold). D: Maximum diastolic voltage. Symbols: V_m = transmembrane voltage; V_{Na} = sodium equilibrium potential; V_K = potassium equilibrium potential; $V_{threshold}$ = threshold voltage; $V_{max\ diastolic}$ = maximum diastolic voltage; absicissa is time (uncalibrated).



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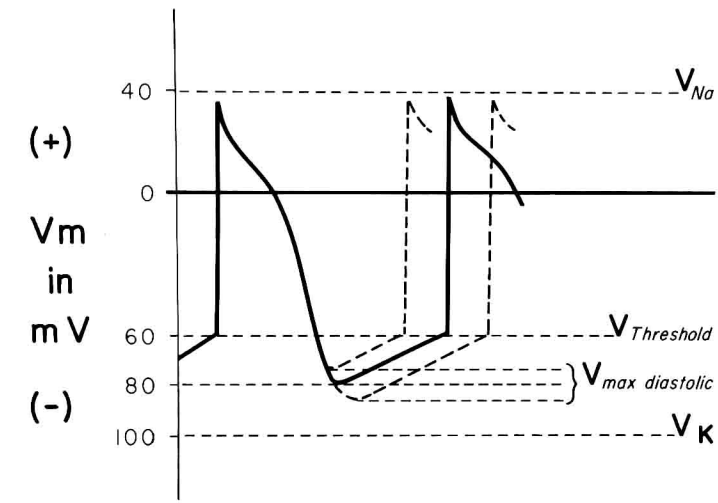

(D)

II. CHARACTERISTICS OF THE "RESTING" MEMBRANE IN CARDIAC CELLS

To understand the genesis of phase 4 depolarization and its expression in the whole heart, it is valuable to understand cardiac cable properties and transmembrane ionic gradients and conductances. The purpose of this section

is to review some of the aspects of these topics which are germane to cardiac automaticity.

A. Membrane Cable Properties

1. Membrane Equivalent Circuit

The cable properties of cardiac cells have been recently reviewed (Freygang and Trautwein, 1970), and the resultant implications concerning cardiac cellular electrical behavior outlined (Bigger and Weld, 1976). Cardiac cable properties have been studied in Purkinje fibers, ventricular muscular trabeculae, and in sinus node (Weidmann, 1952, 1970; Bonke, 1973*a*). The resting Purkinje fiber membrane has electrical characteristics of conductance (reciprocal of resistance) and capacitance which determine the membrane's voltage response to current (charged ion flow). Although it is an oversimplification, the membrane model in Figure 2 illustrates most passive and active electrophysiological characteristics of the membrane. R_1 and R_2 represent the membrane's electrical resistance to ionic current flow owing to the physicochemical structure of the membrane and internal structure of the fiber bundles. For a cardiac cell membrane resting at a voltage substantially negative to the critical firing threshold, R_1 and R_2 may be considered constant for very small (e.g., 1 mV) displacements of transmembrane voltage away from the resting value. However, membrane resistance is determined largely by voltage- and time-dependent ionic conductances, so that R_1 and R_2 cannot be accurately regarded as having static values under conditions of changing transmembrane voltage.

From a morphologic standpoint, R_1 probably resides in the surface membrane across which ionic transfer from perfusing solutions directly into the cell interior can occur. R_2 represents, at least in part, the resistance of the intercellular clefts which separate apposing cardiac cell membranes and lead to the exterior of the multicellular fiber bundles (Falk and Fatt, 1964; Fozzard, 1966; Freygang and Trautwein, 1970). The extensively folded sarcolemmal membrane of individual Purkinje cells and fiber bundles (Mobley and Page, 1972) is probably responsible for the larger capacitance of Purkinje strands per cross-sectional area compared to other cardiac tissue types. C_1 is the capacitance of the sarcolemmal membrane containing R_1, probably located at the outer margins of fiber bundles. C_2 probably represents the capacitance of the more deeply located cell membranes and the membranes lining the deep intercellular clefts.

Capacitance is an inherent property of the surface and cleft membranes, reflecting the ability of the membrane to store electrical charge when subjected to voltage differences across its inner and outer margins. Capacitance