Calcium Blockers

Mechanisms of Action and Clinical Applications

edited by

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Preface

Rarely in the history of science and medicine has a group of pharmacologic compounds greatly influenced more than one or two disciplines either from the point of view of the basic scientist or the clinician. We are fortunate to be witnesses to one such occasion. The group of pharmacologic compounds are the "calcium blockers," agents that affect in one or more ways the normal use of calcium ions by the cell. Since calcium ions are of basic importance to many normal physiologic functions, agents that interfere with calcium activity have the potential of affecting many different physiologic processes. Such agents have tremendous potential, therefore, for application to the study of normal and abnormal calcium-dependent cellular processes as well as for use in treating a variety of disorders. For these reasons, the calcium blockers have aroused the interest of participants in many different basic physiologic as well as clinical disciplines.

Before proceeding, the matter of nomenclature must be addressed. The group of pharmacologic compounds being discussed here has been referred to in several different ways: calcium antagonists, calcium blockers, calcium channel blockers, calcium influx blockers, calcium inhibitors. There has not yet been universal acceptance of a single name that adequately describes these agents as a group. The reason for the confusion is very simple. The group of compounds is quite heterogenous with regard to not only chemical structure but also physiologic effects. Furthermore, the precise mechanism of action of several of these compounds has not been clearly described. Therefore, we have chosen to defer a decision on nomenclature to a later date, when more information has become available. For the purposes of this book, we have simply referred to these compounds as calcium blockers. However, the reader must be aware that this terminology is far from adequate when considering all those agents included in this heterogenous group of drugs.

When we first discussed the possibility of publishing a book on calcium blockers with our colleagues (many of whom have subsequently contributed to this volume), an often-repeated question was "Why another review of calcium blockers?" Over the last several years, there have been approximately ten major reviews of this subject in the form of journal reviews, monographs, and symposiums that have subsequently been published in monograph form. The majority of these reviews, however, have been addressed to the basic science community. In reviewing these reviews, it occurred to us that there existed a rather large information gap between the scientists engaged in basic studies of the mechanisms of action of the calcium blockers and the physicians engaged in preliminary tests of these agents in the clinical setting. Furthermore, since a number of these drugs are just now coming into clinical use, we believe that an important and useful contribution can be made by a comprehensive review of both the basic science information and the recent clinical experience with these agents.

This book, therefore, is directed to three major audiences: the practicing physician, who will require background information when the calcium blockers are fully available for human use; the physician scientist, who requires more basic science information, especially with regard to mechanisms of action

of the calcium blockers at the cellular level; and the basic science investigator, either physiologist or pharmacologist, who is interested in up-to-date and comprehensive reviews of both the basic science and clinical application aspects of the calcium blockers. The book is organized into three major sections. Section I deals primarily with the basic physiology of calcium and provides background information on the normal processes controlling calcium action as well as the effects of calcium ions in those tissues that are, at present, of primary importance in determining the systemic effects of calcium blockers: cardiac muscle, vascular smooth muscle, exocrine pancreas, and endocrine systems. Section II reviews the basic pharmacology of the calcium blockers, emphasizing cardiac and vascular smooth muscle, and introduces the third section by reviewing the clinical pharmacology of these agents. Section III addresses the various applications of the calcium blockers in the clinical setting and the relative effectiveness of these agents for the treatment of a variety of cardiovascular diseases. It is hoped that the combining of these three approaches—all of which are essential to the understanding of the mechanisms of action and the clinical applications of the calcium blockers—will stimulate new ideas for future progress in this most interesting and potentially beneficial area of endeavor.

The editors are grateful to those who have participated in this volume, not only for their efforts (which in all cases have resulted in scientifically and editorially outstanding contributions to the literature) but also for their cooperation in meeting early deadlines. In addition, we would like to acknowledge the secretarial efforts of Nina Kaye Gingerich and Mrs. Carol A. Thormann, without whose assistance this book would not have been possible.

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Section I

Chapter 1

Calcium Ions and Cardiac Electrophysiology

Robert S. Kass and Todd Scheuer

Introduction

Cardiac muscle, like skeletal muscle and nerve, is electrically excitable. Stimulation of a local region by a brief shock of adequate strength generates an impulse which propagates in a regenerative manner to more distant regions. However, in contrast to impulses in other excitable cells, electrical activity in different regions of the heart consists of action potentials of several different shapes. This diversity of action potential configuration reflects the multiple roles of electrical activity in the heart. But despite regional differences, these electrical impulses are generated by membrane permeability changes which generally resemble those in other excitable tissues.

This chapter presents a review of the ionic basis of electrical activity in the heart with an emphasis on the role of calcium ions. The chapter is divided into sections that discuss (1) the principles of impulse generation and conduction in excitable tissue, (2) impulse spread in the heart, (3) the ionic basis of cardiac action potentials, and (4) two inward currents in the heart (in which sodium current is reviewed briefly and calcium current is presented in some detail).

Impulse Propagation in Nerve and Muscle

Local Circuit Currents

Electrical impulses in nerve and muscle are generated by local changes in the relative permeabilities of the surface membranes of these cells to certain ions. These local permeability changes, in turn, regulate the electrical potential difference across the surface membrane through the electrochemical potential differences of the permeant ions. As a consequence of these local changes in membrane potential, voltage gradients are established, which force the movement of intracellular and extracellular ions. This ionic movement constitutes local circuit, or action, currents that spread the impulse from active to passive regions and form the basis of impulse propagation in excitable cells.

The principal membrane mechanisms underlying excitation and propagation of impulses in cardiac cells are similar to those in nerve and skeletal muscle. A brief review of this general process is useful in understanding the contribution of ionic currents to cardiac electrical activity as well as in recognizing some of the special problems involved in their measurement.

The local circuit currents that spread electrical excitation are composed of current loops linking active and passive regions. These loops may be broken down into four branches.

In an *active region*, positively charged ions move down their electrochemical gradients through open channels and constitute an inward transmembrane current. This excitatory inward current, carried by sodium or by calcium ions, adds positive charge to the inner surface of the cell membrane and thus leads to local depolarization.

Intracellularly, a longitudinal voltage difference is established between this local depolarized region and more distant areas which are at rest. This voltage drop forces ions (mostly K^+) to flow intracellularly from active to passive areas. This forms the second branch of the local circuit current loop.

At the *passive region*, current leaks across the surface membrane from inside to outside in the form of displacement current. Thus the charge stored on the membrane capacitance is changed and the region is depolarized. Depolarization, in turn, causes changes in membrane permeabilities, eventually transforming the passive region into a new active region.

The current loop is completed by *extracellular* current (carried by Na^+ or Cl^-) that flows longitudinally from the passive region back to the active region.

Repolarization is conducted in a similar manner, but in this case, the direction of the local current loop is reversed: outward current flows across the surface membrane in the active repolarization region.

The branches of local circuit current loops in heart cells differ from those in other excitable cells only in the intracellular branch. Because of the multicellular nature of heart muscle, this current branch flows through longitudinal resistance that is due in part to the special gap junctions (nexuses) that link adjoining cells. As discussed in detail by Tsien and Siegelbaum (1978), these junctions do not seriously change the mechanism of propagation under normal conditions. Thus, mathematical analysis which assumes a constant, uniform intracellular resistivity may be applied to analyze impulse spread in cardiac tissues.

Cable Theory

The mathematical description of impulse conduction based on local circuit currents in nerve (Hodgkin and Rushton, 1946) is essentially the same formalism originally developed by Kelvin to describe transmission along submerged telegraph cables, and is thus referred to as "cable theory." In addition to describing impulse conduction, cable theory provides the theoretical basis for commonly used methods of measuring current in excitable cells. In this section some of the consequences of one-dimensional current flow are discussed. For the more complicated case of three-dimensional spread, see Jack et al. (1975).

For one-dimensional spread, the cable equation may be written as

$$\frac{a}{2R_a}\frac{\partial^2 V}{\partial x^2} = I_m = I_i + C_m\frac{\partial V}{\partial t}, \quad (1.1)$$

where a is fiber radius, R_a is myoplasmic resistivity, V is membrane potential, I_m is transmembrane current, I_i is ionic current, and C_m is membrane capacity per unit area. This equation simply presents a mathematical summary of the local currents: any changes in longitudinal current (left side of Equation 1.1) must show up as transmembrane current (right side of Equation 1.1). Further, this expression shows that *total* transmembrane current (I_m) consists of two components: ionic (I_i) and capacity ($C_m[\partial V/\partial t]$) currents.

Conduction Velocity

One consequence of this description of impulse spread is a prediction of conduction velocity. Under conditions of propagation at a constant velocity (θ), it can be shown (Noble, 1979) that Equation 1.1 becomes

$$\frac{a}{2R_a\theta^2}\frac{\partial^2 V}{\partial t^2} = I_i + C_m\frac{\partial V}{\partial t}. \quad (1.2)$$

If the ionic current (I_i) is known, this equation defines conduction velocity (θ). Two important characteristics of θ emerge from this description:

(1) θ is proportional to fiber radius and
(2) θ is roughly proportional to the magnitude of the ionic current (see Jack et al., 1975).

These two characteristics are important to the physiologic roles of inward cardiac currents. Large fibers and large excitatory currents are best suited for rapid spread of the impulse, whereas small fibers and small currents result in slowly conducted impulses.

Conditions of Spatial Uniformity: Current Clamp

Simplification of Equation (1.1) can be achieved by experimentally eliminating spatial variation in membrane potential (left side of Equation 1.1). In the squid axon, this can be accomplished by short-circuiting the interior of the axon with an intracellular, low-resistance, axial electrode. Then Equation (1.1) becomes

$$I_i = -C_m \frac{dV}{dt}. \qquad (1.3)$$

Although the multicellular nature of heart muscle precludes the experimental simplification used in nerve, Equation (1.3) can be applied to small isolated cardiac preparations (see below). Then, under conditions approximating zero longitudinal current, Equation (1.3) can be used to estimate net ionic current from time-dependent changes in membrane potential. However, this approach must be used with caution, as it is restricted by frequency-dependent changes in membrane capacity (Fozzard, 1966; Carmeleit and Willems, 1971) as well as nonlinear characteristics of cardiac currents (Strichartz and Cohen, 1978).

Voltage Clamp

A more powerful simplification of Equation (1.1) occurs when membrane potential is measured and experimentally controlled by passing current from an intracellular current source. This technique is referred to as voltage clamp. Since voltage is controlled, capacity current (excluding brief transients) is eliminated, and the total applied current (which is measured) becomes transmembrane ionic current (I_i). Once again this requires uniform voltage control, a condition that can be attained in squid axon with axial wire arrangements but only approximated in multicellular cardiac preparations (see below).

Voltage Clamp of Multicellular Tissue: Limitations

Until very recently, all voltage-clamp experiments in cardiac preparations were carried out in small, isolated cell bundles. Because of their multicellular nature as well as the small size of the individual cells, it is not possible to insert an axial wire in these preparations as is the case in the squid axon. Consequently, uniform voltage control (space-clamp conditions) cannot exist, and voltage-clamp experiments must be carried out under conditions in which spatial and temporal voltage nonuniformity remain within reasonable limits of error. Two voltage-clamp techniques generally have been used in cardiac preparations: the two-microelectrode voltage clamp and the sucrose gap method. Both techniques are subject to the same restrictions due to voltage nonuniformity.

When the passive cable properties of a preparation are known, Equation 1.1 can be used to predict the degree of voltage nonuniformity to be expected for different ionic currents. If the membrane conductance is linear, then closed-form, analytical solutions can be obtained for Equation 1.1 that predict a voltage fall with distance from the current source. The benchmark for measuring voltage decrements is the space constant λ, which is the distance required for voltage to fall to roughly ⅓ its value at the current source. Deck et al., (1964) first showed that voltage control was adequate for studying linear and small outward membrane currents when

preparation lengths were made equal to or less than λ.

If the membrane current-voltage relation is nonlinear, as is the case for most cardiac currents, closed solutions do not exist for Equation 1.1 and the above arguments are not applicable. In particular, as pointed out by Johnson and Lieberman (1971), when inward currents are being studied, instead of falling with distance from the current source, the voltage drop across the membrane actually increases, sometimes resulting in loss of voltage control. Kass et al. (1979) computed theoretical voltage distributions for nonlinear current-voltage relations appropriate for regenerative inward currents in Purkinje fibers. Using this theory, they were able to empirically test the accuracy of voltage clamp measurements of inward currents in cardiac preparations. The results placed limits on the magnitude of measurable inward current. Within these limits, voltage nonuniformities were acceptably small, and under appropriate conditions it was then possible to measure with confidence small inward currents in the heart.

Ionic Currents, Channels, and Gating

Since total membrane current in the heart consists of several ionic components, the experimenter's strategy is to use pharmacologic and kinetic techniques to isolate individual current components. Then, in the simplest cases, each current can be described in terms of a conductance and a driving force,

$$I_i = G_i(E_m,t) \cdot (E_m - E_i), \quad (1.4)$$

where $G_i(E_m,t)$ is a voltage and time-dependent conductance, E_i is the equilibrium potential for a particular channel, and E_m is the membrane potential, which is under experimental control. The difference $(E_m - E_i)$ is the electromotive driving force, which influences the magnitude and direction of ionic movement.

Ions move across membranes through channels that can discriminate between possible charge carriers. The relative permeability of a channel to various ions is referred to as the selectivity of the channel. This characteristic is reflected in the equilibrium potential of Equation 1.4, or the potential of zero *net* current through the channel, which is a function of ionic concentration gradients across the membrane. Thus charge carriers are identified by studies of the dependence of E_i on ion concentrations.

The conductance of a channel can be influenced by the electrical potential difference across the membrane. Some channels open in response to a depolarization; some channels open and then close. The process of opening is referred to as activation, whereas the latter process is known as inactivation (Hodgkin and Huxley, 1952). Macroscopic currents measured across a membrane reflect the total number of channels open at a particular voltage, and changes in these currents reflect changes in the number of open channels induced by variation in membrane potential.

Channel conductance is regulated by the movement of endogenous charged particles within the cell membrane, known as gating particles. Since these particles are charged, they interact with the electric field across the membrane and can move, or undergo conformational change, when this field is changed. Activation occurs when a gating particle is removed from the channel in response to a depolarization of the membrane, leaving a clear path for ion flow. Inactivation occurs when other gating particles enter and block the channel. Questions about the molecular identity of gating particles, as well as possible interrelations between activation and inactivation processes, are presently under investigation.

Normal Spread of the Impulse

The ionic currents generated by these mechanisms in combination with the cable prop-

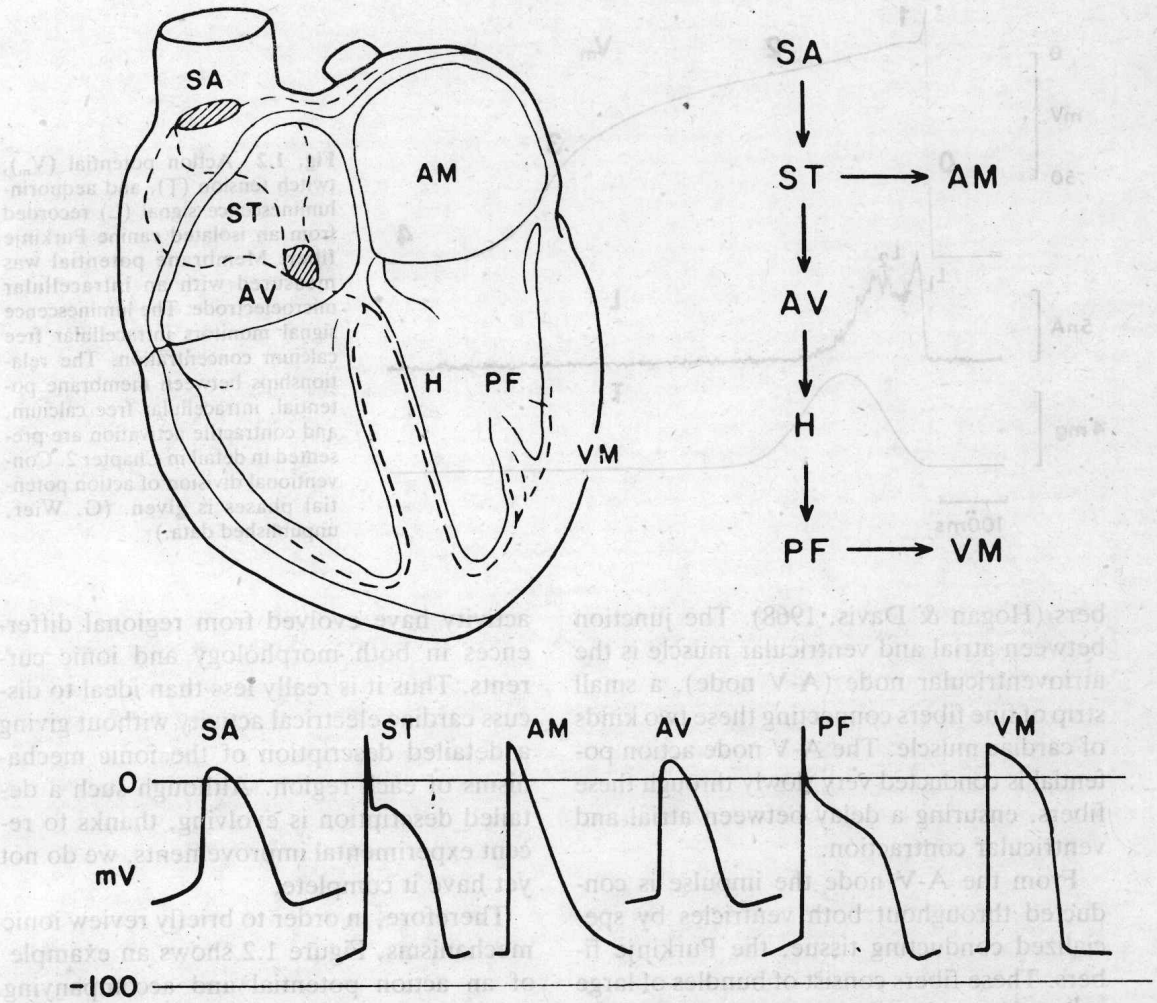

Fig. 1.1 Impulse spread in different regions of the heart. Top: Sequence of impulse spread. Propagation from sinoatrial node (SA) to specialized atrial tracts (ST) and thence to atrial muscle (AM) as well as atrioventricular junction (AV). From A-V junction to Bundle of His (H), then Purkinje fibers (PF) and finally ventricular muscle (VM). Bottom: Action potentials from various regions differ in their duration, shape, and voltage range. (Reproduced with permission from Tsien and Siegelbaum, 1978.)

erties and morphology of different regions of the heart are responsible for the orderly pattern of cardiac excitation and contraction. This pattern is described in the following section.

The sinoatrial (S-A) node is the dominant pacemaker in the heart. Under normal conditions, S-A node fibers spontaneously follow a cyclic pattern of electrical activity, depolarizing and repolarizing at the highest rate in the heart. Impulses which originate in this region, near the junction of the superior vena cava and the right atrium, spread through the heart in an ordered sequence (see Tsien and Siegelbaum, 1978; and, for more detail, Hoffman and Cranefield, 1960). Distinct electrical responses have evolved in different regions of the heart to ensure proper distribution and timing of impulse spread.

The normal sequence of impulse spread is illustrated in Figure 1.1, along with drawings representative of electrical activity in different areas of the heart. The impulse spreads quickly from the S-A node throughout the atrial muscle, aided by special conducting tissue that resembles ventricular Purkinje fi-

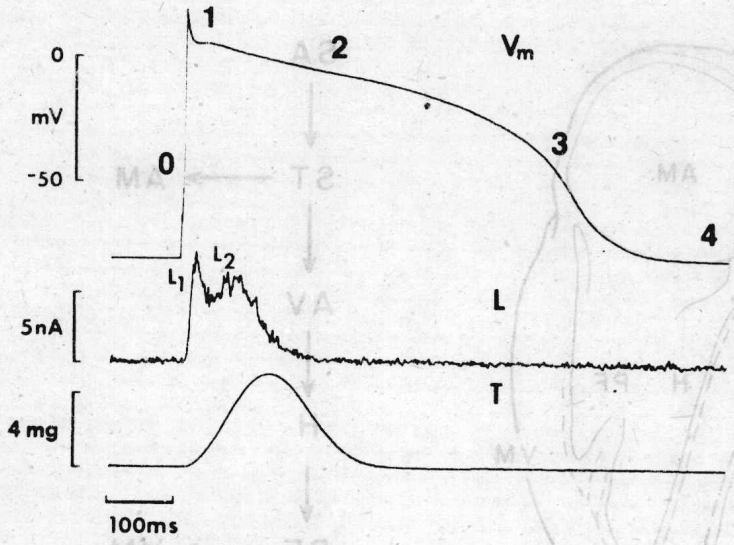

Fig. 1.2 Action potential (V_m), twitch tension (T), and aequorin-luminescence signal (L) recorded from an isolated canine Purkinje fiber. Membrane potential was measured with an intracellular microelectrode. The luminescence signal monitors intracellular free calcium concentration. The relationships between membrane potential, intracellular free calcium, and contractile activation are presented in detail in Chapter 2. Conventional division of action potential phases is given. (G. Wier, unpublished data.)

bers (Hogan & Davis, 1968). The junction between atrial and ventricular muscle is the atrioventricular node (A-V node), a small strip of fine fibers connecting these two kinds of cardiac muscle. The A-V node action potential is conducted very slowly through these fibers, ensuring a delay between atrial and ventricular contraction.

From the A-V node the impulse is conducted throughout both ventricles by specialized conducting tissue, the Purkinje fibers. These fibers consist of bundles of large cells and are therefore well suited for rapid impulse conduction. With this rapid conduction, muscle fibers throughout both ventricles are excited within a few milliseconds and consequently produce a synchronous contraction.

Ionic Basis of Action Potentials in the Heart

Action potentials recorded from different regions of the heart may be distinguished by electrical characteristics that are well correlated with the regional physiological roles of the impulse. The differences in electrical activity have evolved from regional differences in both morphology and ionic currents. Thus it is really less than ideal to discuss cardiac electrical activity without giving a detailed description of the ionic mechanisms of each region. Although such a detailed description is evolving, thanks to recent experimental improvements, we do not yet have it complete.

Therefore, in order to briefly review ionic mechanisms, Figure 1.2 shows an example of an action potential and accompanying twitch tension recorded from an isolated calf Purkinje fiber. The figure shows the conventional division of different phases of the action potential, and the ionic currents associated with each phase are listed in Table 1.1. The roles of these currents in the different action potential phases resemble those of ionic currents in other cardiac fibers. Differences arise, perhaps, in the relative amounts of various currents or in the total number of time-dependent currents in a particular region.

Each of these Purkinje fiber membrane currents is described in detail by Noble (1979) and in an earlier paper by McAllister et al. (1975), although several characteristics of these currents have been updated since these articles were written. Instead of describing all of these currents, the remainder of this chapter will focus on the two regenerative

Table 1.1 Major Time-dependent Membrane Currents in the Purkinje Fiber.

Action potential phase	Membrane current	Kinetic characteristics	Principal charge carrier
Rapid upstroke (phase 0)	Excitatory inward (I_{Na})	Very fast activation fast inactivation	Sodium ions
Fast repolarization (phase 1)	Transient outward current (I_{to})	Rapid activation slower inactivation	Potassium ions (this current is, at least in part, a Ca-activated potassium current)
Plateau (phases 2 and 3)	The plateau is maintained by a balance of two time-dependent currents: 1) slow inward current (I_{si}) 2) delayed rectifier (I_x)	I_{si}: rapid activation slow inactivation	I_{si}: calcium ions
		I_x: slow activation *no* inactivation	I_x: largely potassium ions
Pacemaker depolarization (phase 4)	Pacemaker current (I_{K2})	Slow activation no inactivation	Potassium ions (in part)

inward currents of the Purkinje fiber and other cardiac tissues.

Two Inward Currents in the Heart

From Equation 1.3, it is clear that inward transmembrane ionic current (positive charges moving from outside to inside the cell) depolarizes the cell membrane, and that the rate of depolarization is proportional to the magnitude of the current. From records like those in Figure 1.1, action potentials in the heart may be divided into two general groups based on rate of depolarization. In one group (atrial and ventricular muscle, Purkinje fibers), the action potentials are characterized by very rapid (500 v/sec) upstrokes, whereas in the second group (S-A and A-V nodal fibers) the action potential rises with a markedly slower upstroke (10 v/sec). Using Equation 1.2 as a guide, this contrast in upstroke velocity suggests that the regenerative inward current in the nodes might be much smaller than in other cardiac tissues, and thus might be carried via a distinct mechanism. Another distinction between these groups is the voltage range from which these upstrokes emerge. The nodal tissues are activated at a considerably more positive voltage than the other cardiac preparations.

These two observations provide clues to the existence of two inward currents in cardiac cells, distinguishable both by the voltage at which they activate and by the differences in their amplitude. Considerable experimental evidence now supports this view and provides several criteria for distinguishing these excitatory currents. One current, carried by sodium ions, resembles regenerative sodium current in nerve and skeletal muscle and is responsible for the rapid upstroke and fast impulse conduction in atrial and ventricular muscle as well as in the special conducting tissue. A second current, carried principally by calcium ions, generates the nodal upstroke, underlies slow impulse conduction in the A-V node, and maintains the plateau phase of the action potential in other parts of the heart. The characteristics of these currents and some of the roles they play in normal and abnormal impulse conduction are described in the following sections.

Sodium Current

The upstroke of the action potential of working cardiac muscle and cells of the specialized conducting tracts is due to a transient increase in membrane permeability to sodium ions. The very rapid rates of rise of the action potentials in these tissues (on the order of 500 v/sec) indicate, from Equation 1.3, that the current responsible for these voltage changes is large (about 1 mA/cm^2).

Sodium currents (I_{Na}) have now been measured in several cardiac preparations (Colatsky & Tsien, 1979; Lee et al., 1979; Colatsky, 1980; Ebihara et al., 1980). In many aspects these currents appear similar to sodium currents in nerve and skeletal muscle. When the cell membrane is depolarized beyond −65 mv, an inward current is initiated which rises to a peak and then declines within a few milliseconds (Fig.1.3A). As in other excitable cells, sodium current in the heart is described as ion movement through channels which are regulated by time- and voltage-dependent activation and inactivation gates. At a particular time and voltage, the fraction of open sodium channels is determined by the product of the activation and inactivation gating parameters. The steady-state voltage dependence of these parameters obtained in the rabbit Purkinje fiber is given in Figure 1.3B.

As this product is zero at all potentials in Figure 1.3B, these curves predict that the sodium conductance must also be zero in the steady-state. However, the data in these curves were obtained at low temperatures. At more physiologic temperatures there is a region of overlap of these two parameters that results in a "window" of steady-state sodium current over the plateau potential range (Attwell et al., 1979). This current ac-

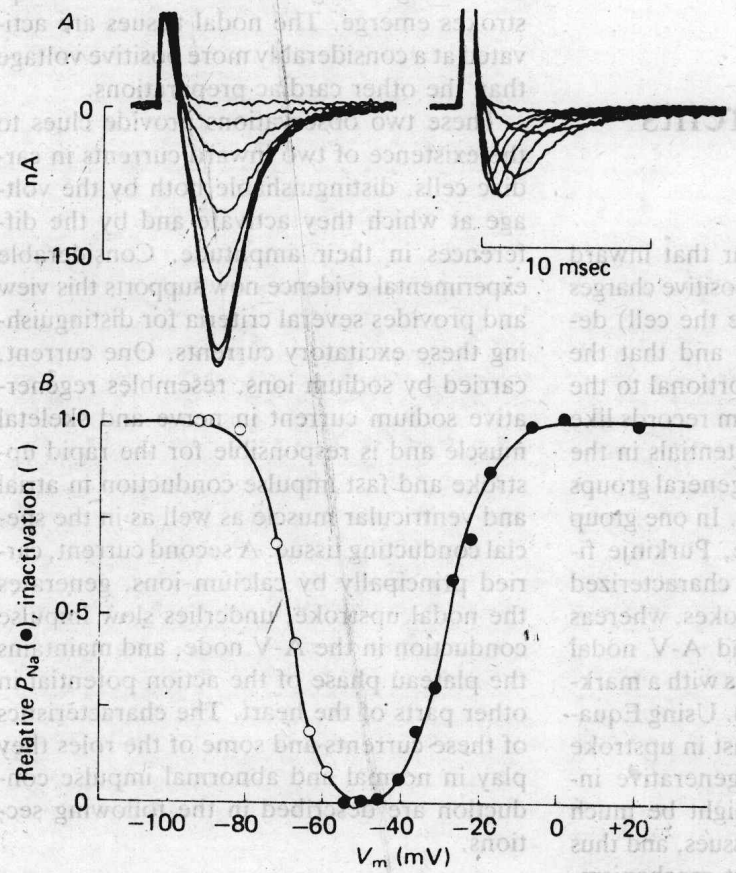

Fig. 1.3 Sodium current in the rabbit Purkinje fiber. *A*. Families of total membrane current obtained using conventional protocols for inactivation (left) and permeability (right). The inactivation curve was measured at a constant test pulse voltage (-38 mV) following 500 msec prepulses. Permeability was measured from peak inward currents measured at various test pulses from a -58 mV holding potential. *B*. Voltage-dependence of relative sodium current inactivation (○) and permeability (●). (Data from Colatsky, 1980.)

counts for the effects of tetrodotoxin (TTX) and sodium removal on the cardiac action potential plateau (Weidmann, 1955; Dudel et al., 1966; Coraboeuf et al., 1979).

Membrane potential affects the inactivated sodium channels, and thus determines the number of sodium channels available for impulse conduction. In the plateau potential range (Fig. 1.3B) most of these channels are inactivated. The time course of removal of sodium channel inactivation at diastolic potentials determines the availability of these channels for impulse conduction and consequently the periods in which the cells remain inexcitable (refractory).

Sodium channel inactivation is also important to the mode of action of local anesthetics. The position of the inactivation curve along the voltage axis is sensitive to these drugs as well as to change in extracellular divalent cation concentration. One mechanism by which these compounds exert their stabilizing effect on membranes is by shifting the sodium current inactivation curve in the negative direction (Lee et al., 1981a; Bean et al., 1981). Thus fewer sodium channels are available at diastolic potentials, and the membrane must be subjected to stronger depolarizations to initiate impulses.

Sodium channels in the heart thus resemble sodium channels in other excitable cells in their voltage dependence and sensitivity to local anesthetic drugs. But some clues—such as a distinct, low TTX sensitivity—suggest that cardiac sodium channels may display marked differences from those of other cells. This interesting possibility is one example of the important question presently being investigated using voltage-clamp analysis of sodium currents in the heart.

Calcium Current

Action Potential Experiments

Under a variety of experimental conditions which result in membrane depolarization, the action potential of the Purkinje fibers or of working myocardial muscle can be made to resemble electrical activity of the S-A or A-V node. This experimentally induced action potential is characterized by a very slow rate of rise (about 10v/sec) and a slow conduction velocity (.1 – .01 m/sec). Consequently, it is referred to as a slow response action potential (Cranefield, 1976, 1977).

In contrast to the normal upstroke of myocardial fibers (or of Purkinje fibers), the slow response upstroke is not abolished by TTX or by removal of extracellular sodium. Instead, it is sensitive to extracellular calcium and other divalent cations, and is inhibited by a group of compounds which do not significantly block sodium channels. The ionic and pharmacologic evidence suggests that this current is distinct from the current carried by sodium channels.

The current which generates the slow response is now referred to as the slow inward current (I_{si}). It is carried principally by calcium ions and is as small as 1/100 of peak sodium currents. In addition to the slow response, it underlies impulse conduction in nodal tissue (Noma et al., 1977; Brown and DiFrancesco, 1980) and is crucial to maintenance of the plateau phase of action potentials of myocardial and special conducting tissues (McAllister et al., 1975; Beeler and Reuter, 1977).

As early as 1956 (Coraboeuf and Otsuka), observations on the action potential of the guinea pig ventricle were difficult to reconcile with only one (sodium-dependent) inward current in the heart. But it was a series of studies on slow responses in Purkinje fibers that provided the clearest evidence for I_{si} (reviewed by Carmeliet, 1980; Reuter, 1979). These results have been confirmed and examined in more detail in voltage-clamp investigations.

Voltage-Clamp Studies of I_{si}

Voltage-clamp investigations in Purkinje fibers (Reuter, 1967; Vitek and Trautwein, 1971) and other preparations (Rougier et al., 1969; Beeler and Reuter, 1970; New and Trautwein, 1972) soon demonstrated the ex-

istence of a calcium-sensitive inward current (I_{si}) contributing to net membrane currents measured positive to −50 mv. This current has many of the properties suggested by the slow response experiments: it is enhanced by catecholamines and blocked by Mn^{++} and other metallic cations, and it has a voltage dependence distinct from I_{Na}.

Detailed studies of I_{si} have been difficult to carry out in Purkinje fibers, as the inward current is masked by two overlapping outward currents in this preparation. The first of these is a transient current which dominates early periods of voltage depolarizations (I_{to}; see Table 1.1); the second is the slowly activating delayed rectifier (I_x), also in Table 1.1, which contributes to currents measured at later times during voltage-clamp steps.

However, even in preparations in which outward current overlap is not so severe (for example, ventricular muscle), studies of I_{si} remained controversial because of the problems which arise when voltage-clamping inward current in multicellular tissue. This difficulty was partially resolved when theoretical calculations defined conditions that permit adequate measurement of I_{si}. Using these conditions, several properties of I_{si} have been established and subsequently confirmed in different cardiac preparations (Table 1.2).

I_{si} has been analyzed by Reuter, Trautwein and others (see Reuter, 1979, for review) and is described analagously to I_{Na} as current through a gated channel.

Its parameters are defined as follows:

$$I_{si} = \bar{g}_{si} \cdot d \cdot f \cdot (E_m - E_{si}), \quad (1.5)$$

Where $\bar{g}_{si}$ is the conductance when all the I_{si} channels are fully open, d is the activation gating variable, f is the inactivation variable, and $(E_m - E_{si})$ is the electrochemical driving force. The gating parameters d and f reflect the fraction of open channels at a given voltage and account for the voltage-dependent opening and closing of this channel. However, the voltage range and time course of the I_{si} parameters are different from those of I_{Na}.

Steady-state Activation and Inactivation. Figure 1.4A shows the measurement of I_{si} using a standard voltage-clamp protocol and a technique which permits a pharmacologic dissection of this current (discussed below). A representative plot of the voltage-dependence of the steady-state values of the activation and inactivation parameters is also

Table 1.2 Measurements of I_{si} in Cardiac Preparations.

Species/Preparation Type	Blocked by: Mn^{++}/D600	TTX	Reference
Ventricular Muscle			
canine trabeculae, papillary muscle	yes	n/a	Beeler and Reuter, 1970
cat trabeculae and papillary muscle	yes	n/a	McDonald and Trautwein, 1978
chick embryonic cell cluster	yes	no	Nathan and DeHaan, 1979
single rat heart myocytes	yes	no	Isenberg and Klöckner, 1980
single guinea pig and rat myocytes	n/a	no	Lee et al., 1981b
Atrial Muscle			
frog sinus venosus	yes	no	Rougier et al., 1969
frog sinus venosus	yes	no	Brown et al., 1977
single frog atrial myocytes	yes	no	Giles and Hume, 1981
S-A Node			
rabbit	yes	no	Noma et al., 1977
rabbit	yes	no	Brown and DiFrancesco, 1980
Purkinje Fiber			
sheep	n/a	n/a	Reuter, 1967
calf	yes	n/a	Kass and Tsien, 1975
calf	yes	n/a	Kass and Wiegers, 1982

shown (Fig. 1.4B). As suggested by the slow response data, the voltage range of I_{si} inactivation is different from that of I_{Na}. The calcium current is activated over potentials at which I_{Na} is almost completely inactivated.

As seen in the current trace of this figure, these channels open (activate) rapidly on depolarization and then close (inactivate) more slowly. Typical time constants for inactivation (τ_f) range from 200 to 500 msec, whereas the activation time constant (τ_d) is reported to reach a maximum of 35 msec (Beeler and Reuter, 1977). Recent experiments that have permitted better resolution of the kinetics of activation suggest that this channel opens much faster than had been suggested by previous data.

I_{si} Channel Selectivity and Sensitivity to Neurotransmitters. The I_{si} equilibrium, or reversal, potential (E_{si}) is determined by measuring the membrane potential at which zero net current flows through the open I_{si} channel. Measurements of changes in the reversal potential when the ionic environment of the channel is systematically varied determine the channel selectivity. Reuter and Scholz (1977a,b) varied extracellular ion concentrations and showed that this channel is highly, but not specifically, selective for calcium ions. They found that sodium or potassium ions also move through the channel, but are approximately 1/100 as permeant as calcium. More detailed measurements of ion permeation through these calcium channels are now being carried out under conditions in which the ionic concentrations on both sides of the cell membrane are controlled (Lee et al., 1981b).

I_{si} is very sensitive to neurotransmitters. It is decreased by muscarinic agents (Giles and Noble, 1976; Ten Eick et al., 1976) and it can be increased by as much as a factor of 2.5 by adrenergic compounds (Kass and Wiegers, 1981). Reuter and Scholz (1977b) showed that the principal action of epinephrine on I_{si} is to increase the maximal conductance ($\bar{g}_{si}$) of this current. This can occur either by increasing the number of calcium channels, each with a constant single-channel conductance, or by increasing the conductances of individual channels while the total number of available channels remains constant. Reuter and Scholz (1977b) suggest the former case, but this must be confirmed by actual measurements of the effects of epinephrine on the I_{si} single-channel conductance.

Recent Experimental Advances in I_{si} Measurement

Several new experimental techniques have been introduced which are providing a clearer and more detailed description of cardiac calcium current. Some of these techniques are outlined here.

Pharmacologic Dissection of I_{si}. The inability to isolate I_{si} from total membrane current records has limited quantitative analysis of this current. This problem is most apparent in the Purkinje fibers, where two time-dependent outward currents overlap I_{si}. Two pharmacologic procedures have been reported to block these outward currents and to unmask I_{si}. The first involves replacement of intracellular potassium by Cs^+ using the iontophore nystatin (Marban, 1981). In a second approach, the quaternary ammonium compound tetrabutylammonium, a potassium blocker in nerve, is injected intracellularly using micro-iontophoresis (Kass et al., 1981). The records in Figure 1.4A were obtained following the latter treatment.

These techniques provide much better resolution of the time course of I_{si} and, in addition, they will permit pharmacologic dissection of this current using calcium blocking agents. Using this approach, Marban and Tsien (1981) have already found that inactivation of the calcium channel might be facilitated by intracellular calcium ions.

Voltage Clamp of Isolated Cardiac Cells. Several groups have reported successful measurements of ionic currents in enzymatically dissociated single heart cells (Lee et al., 1979; Isenberg and Klöckner, 1980;

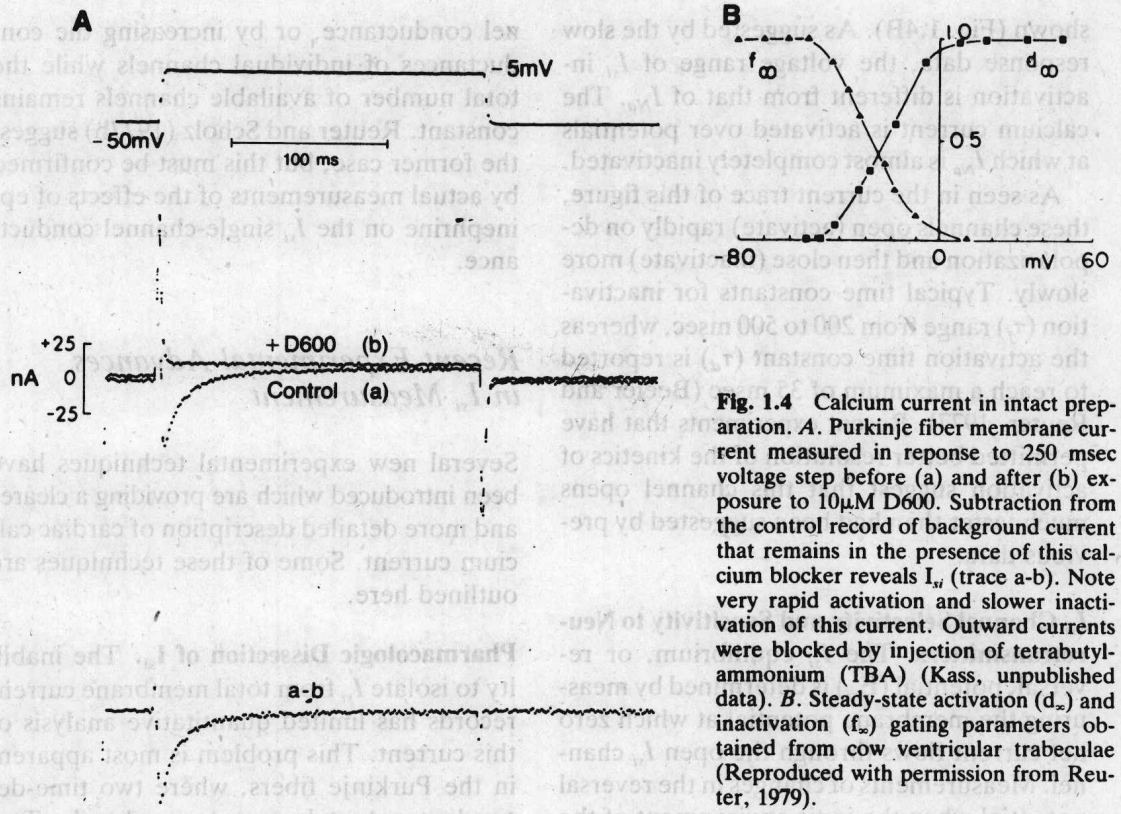

Fig. 1.4 Calcium current in intact preparation. *A*. Purkinje fiber membrane current measured in reponse to 250 msec voltage step before (a) and after (b) exposure to 10μM D600. Subtraction from the control record of background current that remains in the presence of this calcium blocker reveals I_{si} (trace a-b). Note very rapid activation and slower inactivation of this current. Outward currents were blocked by injection of tetrabutylammonium (TBA) (Kass, unpublished data). *B*. Steady-state activation (d_∞) and inactivation (f_∞) gating parameters obtained from cow ventricular trabeculae (Reproduced with permission from Reuter, 1979).

Hume and Giles, 1981). This approach avoids the problems associated with voltage-clamp studies of multicellular preparations and provides the best method for studying channel parameters with very fast kinetics. An example of calcium current recorded from single atrial cells is given in Figure 1.5, and a summary of cardiac preparations in which this current has been measured is given in Table 1.2.

Calcium Blockers: Roles as Biophysical Tools and Therapeutic Agents

Calcium current in each of these preparations is inhibited by the same, rather diverse group of compounds, which includes the metallic cations La^{++}, Mn^{++}, Co^{++}, Ni^{++}, and several organic agents such as D600, verapamil, and nifedipine (these agents and their effects in cardiac muscle are discussed in more detail in Chapter 10). These blockers have great potential in the treatment of calcium-dependent electrical disturbances in the heart. In addition they can be very useful in biophysical studies of I_{si}.

Pharmacological Dissection of I_{si}

Selective block of I_{si} is very desirable from the point of view of the electrophysiologist, as that would permit its dissection from other currents that are measured in response to voltage clamp test pulses. The best example of successful application of this procedure is the use of TTX to isolate sodium currents in nerve.

This procedure requires high specificity of the I_{si} blocking compound. Unfortunately, all I_{si} blockers investigated to date have been shown to affect the two outward plateau currents I_{to} and I_x in addition to I_{si} (Kass and