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Delayed Afterdepolarizations in Heart Muscle: Mechanisms and Relevance*,†

CRAIG T. JANUARY AND HARRY A. FOZZARD‡

Cardiac Electrophysiology Laboratories, Departments of Medicine (Cardiology) and Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois

I. Introduction

THE CELLULAR MECHANISMS that cause cardiac arrhythmias are of immense importance and are the object of intense investigation. One mechanism postulated to cause cardiac arrhythmias, and possibly conduction disturbances, is delayed afterdepolarizations (also called late afterdepolarizations, oscillatory afterpotentials, or transient depolarizations). These depolarizations are induced by Ca^{2+} overload of the cardiac cell. In the last decade, several review articles have been published on the subjects of delayed afterdepolarizations and of mechanisms of arrhythmias (16, 28, 86). The purpose of this review is to summarize recent experimental evidence pertinent to the mechanisms responsible for delayed afterdepolarizations and their relation to Ca^{2+}, and to gain insight into their clinical relevance.

II. Induction of Delayed Afterdepolarizations

Delayed afterdepolarizations are oscillations of membrane voltage that occur after complete repolarization of the cardiac action potential. Hence, they are initiated during electrical and mechanical diastole. Delayed afterdepolarizations are a type of triggered activity. They do not occur spontaneously in unstimulated preparations; rather, their induction requires an initiating or triggering event such as one or more action potentials. The amplitude of a delayed afterdepolarization can be subthreshold (i.e., not reaching threshold voltage and initiating an action potential), or a delayed afterdepolarization can reach threshold voltage and result in an action potential. When threshold is achieved repetitively, sustained rhythms can be produced under certain experimental conditions in several cardiac cell types (see ref. 86 for review).

The prototypical experimental method used to induce delayed afterdepolarizations is to expose cardiac tissue to higher concentrations of cardiac glycosides. This is not a property of a particular cardiac glycoside, since in the same preparations and under similar experimental conditions several cardiac glycosides have been shown to induce delayed afterdepolarizations (39). Cardiac glycosides are known to inhibit the Na-K exchange pump, causing the intracellular Na^+ activity to rise (18, 51, 83). Through the Na-Ca exchange mechanism this results in a rise in intracellular Ca^{2+} and the development of tension (51, 74). Consistent with a role for intracellular Ca^{2+} in delayed afterdepolarizations, a transient contraction (the aftercontraction) can be recorded concomitant with delayed afterdepolarizations. Delayed afterdepolarizations in cardiac cells have been attributed to Ca^{2+} overload, which can then result in a damped oscillatory release of Ca^{2+} from internal stores. In agreement with this hypothesis, several other interventions that raise intracellular Ca^{2+} by different mechanisms also have been shown to enhance development of delayed afterdepolarizations or the underlying transient inward transmembrane current (see below). These interventions in-

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‡ Address reprint requests to: Harry A. Fozzard, M.D., Box 440, Department of Medicine (Cardiology), The University of Chicago, 5841 South Maryland Ave., Chicago, IL 60637.

clude lowering or removing $[K]_o$,[§] lowering $[Na]_o$, raising $[Ca]_o$, and exposing the tissue to catecholamines.

III. A Transient Inward Current Causes Delayed Afterdepolarizations and Is Induced by Intracellular Ca^{2+}

In voltage-clamped cardiac preparations (40, 50) and single cells (60, 62), a transient inward current (i_{TI}) has been associated with delayed afterdepolarizations. Several lines of evidence suggest that i_{TI} is the current that underlies delayed afterdepolarizations and that it is closely linked to a rise in intracellular Ca^{2+}. (*a*) The appearance of i_{TI} coincides temporally with the development of delayed afterdepolarizations and aftercontractions following exposure to cardiac glycosides, and they all appear at similar concentrations. (*b*) The dependence of delayed afterdepolarizations and i_{TI} on frequency of stimulation is similar (50, 81). (*c*) Ca^{2+} overload of heart cells results in increased spontaneous membrane voltage or current (recorded under voltage clamp conditions) noise. Power spectral analysis has shown that the frequency distribution contained within these voltage or current signals is similar (42, 60), and that evoking i_{TI} results in additional power that contains the same frequency distribution (42). A similar relationship has been shown between the frequency spectra of spontaneous membrane current noise and tension fluctuations (42; see also ref. 10). These and other studies (41) showed that changes in contractile force lagged behind changes in membrane current in a voltage-dependent manner by 40 to 140 ms. (*d*) An intracellular Ca^{2+} transient has been associated with delayed afterdepolarizations (84). (*e*) Intracellular injection of Ca^{2+} elicits delayed afterdepolarizations (60). (*f*) Modification of intracellular Ca^{2+} by the injection of ethyleneglycol-bis(β-aminoethylether)-N,N′-tetraacetic acid (EGTA) into cells, or by the application of caffeine, suppresses both delayed afterdepolarizations and i_{TI} (60; see also ref. 42). Ryanodine, which blocks Ca^{2+} release from the sarcoplasmic reticulum (SR), has been shown to suppress delayed afterdepolarizations or i_{TI} and the associated Ca^{2+} transient (59, 76, 79). Recently, it was shown that, when 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), a potent chelator of Ca^{2+}, was diffused into cardiac tissue, it also abolished delayed afterdepolarizations, the associated oscillation in intracellular Ca^{2+}, and the aftercontraction (59).

IV. Calcium Release by the Sarcoplasmic Reticulum

The SR of striated muscle is the major storage site for the Ca^{2+} that is released to produce normal contraction (44). First, the action potential activates a small Ca^{2+} current. Ca^{2+} for contraction is then released by the SR, and it diffuses to the myofibrils. Binding to troponin C initiates cell shortening or force development through interaction of actin and myosin filaments. The released Ca^{2+} is then pumped back into the SR by an ATP-dependent Ca^{2+} pump and stored for the next contraction.

The Ca^{2+} uptake phase of SR function has been well studied. The primary amino acid sequence of the Ca^{2+} pump has been determined, and much of its 3-dimensional structure deduced. However, the Ca^{2+} release phase of SR function is less well understood. The major hypothesis to explain Ca^{2+} release from cardiac SR is that release is triggered by an increase in cytoplasmic Ca^{2+} (25–27; see also ref. 4). According to this concept, transsarcolemmal Ca^{2+} current increases Ca^{2+} in the vicinity of the SR, causing it to release its store. With an action potential as trigger, release of SR-stored Ca^{2+} under normal conditions is complete and is sufficient for a contraction that is 10 to 20% of maximum. Additional Ca^{2+} loading of the cell, and consequently of the SR, can therefore result in a 5- to 10-fold increase in contraction strength. Partial release of Ca^{2+} stored in the SR can be obtained experimentally, and this may occur naturally under unusual circumstances. The mechanism of Ca^{2+}-triggered release is not well understood, but there is recent evidence that the SR of cardiac muscle contains Ca^{2+} channels that are gated by Ca^{2+} (72).

The cytoplasmic Ca^{2+} level itself is established by a complex interaction between sarcolemmal influx through Ca^{2+} channels and perhaps Na-Ca exchange, efflux through Na-Ca exchange and the sarcolemmal Ca^{2+} pump, intracellular sequestration in the SR and perhaps in organelles such as mitochondria, and binding to troponin C and perhaps to other cytoplasmic molecules. When the resting myocardial cell is overloaded with Ca^{2+}, the SR cannot maintain its increased store, and spontaneous release can be seen, even in quiescent cells (1, 23, 49, 85). Spontaneous fluctuations can then occur in the membrane potential, the membrane current under voltage clamp, and contractions (42). The relation between spontaneous or cyclical Ca^{2+} release and the Ca^{2+} transient producing the afterdepolarization is proposed to be the following. If the cell is stimulated to have action potentials and contractions, the SR releases its Ca^{2+} synchronously, and it reaccumulates Ca^{2+} in a similarly synchronous fashion. When overfilled with Ca^{2+}, the SR can then spontaneously release Ca^{2+} again. This spontaneous release and reaccumulation may occur for 2 or 3 cycles, until Ca^{2+} reassumes steady-state conditions or release becomes asynchronous. The rise in Ca^{2+} temporally correlates with the aftercontraction that characterizes the Ca^{2+}-overloaded state (66, 84).

The mechanism of this spontaneous release in Ca^{2+}-overloaded SR is not yet determined. It could result from

§ Abbreviations used are: $[K]_o$, $[Na]_o$, and $[Ca]_o$, extracellular K, Na, and Ca concentrations, respectively; i_{TI}, transient inward current; EGTA, ethyleneglycol-bis(β-aminoethylether)-N,N′-tetraacetic acid; SR, sarcoplasmic reticulum; Ca_i, intracellular Ca; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; TMA, tetramethylammonium; TRIS, Tris(hydroxymethyl)aminomethane.

"breakdown" of the membrane, backward transport through the Ca^{2+} pump, or opening of the SR Ca^{2+} channels. The most likely mechanism would seem to be opening of SR Ca^{2+} channels. It also has been shown that increasing cytoplasmic Ca^{2+} in "skinned" cardiac cells (25) can produce cyclical Ca^{2+} release from SR. The presence of spontaneous release and reaccumulation that can be seen in isolated SR or in skinned cells is evidence that sarcolemmal current is not required to trigger the cyclical events, although Lin et al. (54) and Boyette et al. (5) have reported a complex relationship between Ca^{2+} current and the occurrence of i_{TI}.

V. The Charge-carrying Mechanism for i_{TI}, Membrane Channel versus Exchange Pump Current

The cellular basis for i_{TI} has remained controversial, and two different charge-carrying mechanisms for i_{TI} are presently hypothesized. One hypothesis (41–43) favors a nonselective cation membrane channel with its conductance regulated by intracellular Ca^{2+}. Under conditions of Ca^{2+} overload, there could occur a cyclical release of Ca^{2+} from the SR (synchronized by the action potential or its repolarization), and this results in a transient increase in the nonselective cation-permeable channel conductance, in parallel with activation of the aftercontraction. In voltage-clamped cardiac Purkinje fibers, Tsien and his colleagues (43) found the reversal potential for i_{TI} to be approximately −5 mV in normal Tyrode's solution. In those experiments, the identification of i_{TI} as a Ca^{2+}-activated current was supported by the simultaneous recording of aftercontractions. Evidence that the current resulted from the Ca^{2+} release, rather than vice versa, included the presence of aftercontractions near the reversal potential of i_{TI}. The reversal potential was sensitive to withdrawal of Na^+ from the bath (becoming about −35 mV), but it was insensitive to replacement of chloride by an impermeant anion. Although changes in the reversal potential with small manipulations of extracellular K^+ (1 to 8 mM) could not be shown, a K^+ permeability (approximately equal to that of Na^+) was required to account for the value of the reversal potential in normal Tyrode's solution (assuming that the only current flow was through the i_{TI} channel). Permeability to Ca^{2+} also was suggested because the reversal potential remained well positive to the K^+ reversal potential in the absence of Na^+ (see also ref. 10). The simplest explanation for these data, based primarily on the identification of a reversal potential for i_{TI}, was to postulate a Ca^{2+}-activated membrane channel with significant permeabilities to sodium, potassium, and calcium ions.

With the development of single channel recording techniques (31), recordings in cardiac cells of a nonselective cation channel activated by intracellular Ca^{2+} have been obtained by Colquhoun et al. (15) in cultured rat neonatal myocytes and Ehara et al. (21) in adult guinea pig ventricular cells. These channels have little selectivity among monovalent cations, but are highly selective against anions, and their gating shows little dependence on membrane voltage. The unit conductance of the channels studied by Colquhoun et al. (15) was 30 to 40 pS (25–27°C), and these channels could be activated by inside Ca^{2+} concentrations of 1 μM. The channels studied by Ehara et al. (21) had a lower unit conductance of about 15 pS (20–25°C). The Ca^{2+} concentration threshold for channel activation was 0.3 μM, and the open probability was half-maximal at a Ca^{2+} concentration of 1.2 μM. Several additional reports of Ca^{2+}-activated nonselective cation channels have appeared for noncardiac tissue beginning with Yellen (88) in neuroblastoma cells (see ref. 21 for references). Hill et al. (34) have reported the occasional incorporation into membrane bilayers of a nonselective cation channel from sarcolemmal vesicles prepared from adult canine ventricular muscle. This channel responded to increased Ca^{2+} by increased probability of being open. The single channel conductance was 120 pS, and the channel opening was markedly voltage dependent. While it is interesting to speculate that this incorporated channel is related to the channels seen by Colquhoun et al. (15), Ehara et al. (21), Yellen (88), and others in intact cells, it must be noted that the properties of the channel found by Hill et al. (34) differ in several important ways. In summary, a nonselective cation channel activated by intracellular Ca^{2+} would explain the experimental data qualitatively, although discrepancies remain in the measured reversal potentials under different ionic conditions. Membrane channels with similar properties have been recorded by patch clamp of heart cells and by the incorporation of sarcolemmal vesicles into lipid bilayers.

The second mechanism suggested for i_{TI} is the electrogenic Na-Ca exchange pump driven by the transmembrane electrochemical gradients for Na^+ and Ca^{2+} ions (2, 43, 65). In the normally polarized cell, Na^+ entering via the exchanger will be coupled to Ca^{2+} extrusion. The stoichiometry for charge translocation is now generally accepted to be 3:2 (i.e., 3 Na^+ to 1 Ca^{2+}; see refs. 46 and 69). The initiating sequence for the delayed afterdepolarization, as with the nonselective cation channel hypothesis, is Ca^{2+} overload, producing cyclical release of Ca^{2+} from the SR and giving rise to an oscillation in myoplasmic Ca^{2+} and the aftercontraction. An oscillatory reduction in the transmembrane Ca^{2+} gradient would facilitate Ca^{2+} extrusion and Na^+ entry by the exchanger. In turn, because of the electrogenicity of the Na-Ca exchange mechanism, this would result in a transient increase of the net inward movement of positive charge (3 Na^+ in for 1 Ca^{2+} out), thereby producing i_{TI}, or a delayed afterdepolarization. It is essential to recognize that the Na-Ca exchange mechanism itself must have a reversal potential at some voltage, and positive to this voltage Ca^{2+} will be transported into the cell and Na^+ will be transported out of the cell (see refs. 22 and 64).

However, at a fixed transmembrane voltage the effect of an oscillatory increase in intracellular Ca^{2+} will be to alter transiently the current in a net inward direction.

The argument favoring the electrogenic Na-Ca exchange mechanism has been supported by several reports of the absence of an experimentally detectable reversal potential for i_{TI}. Arlock and Katzung (2) studied ouabain-intoxicated papillary muscles using a sucrose gap voltage clamp technique and found that the amplitude of i_{TI} became progressively smaller at less negative voltages, but it did not reverse its polarity at voltages up to +30 mV. One possibility they suggested for the apparent reversal potential found previously by other investigators was that it could arise from Ca^{2+}-dependent activation of i_{TI} channels to carry outward current. Another possibility is suggested by the recent finding of Ca^{2+}-activated outward K^+ channels in Purkinje cells (9). In sinoatrial node tissue, a current resembling i_{TI} can be induced by exposure to low $[K]_0$ solutions (7). Near voltages where a nonselective cation membrane channel mechanism might reverse its polarity, low amplitude current oscillations persisted. The timing of the current oscillations was voltage dependent, which complicated the differentiation of inward from outward transient components. Vassalle and coworkers (54) studied Purkinje fibers and also reported failure of i_{TI} to reverse its polarity. Unfortunately, the range of voltages in their study was limited to negative potentials. In embryonic heart cell aggregates, a transient inward current resembling i_{TI} is induced with abrupt exposure to caffeine (13). At less negative potentials, its amplitude was decreased, but it failed to reverse polarity at potentials up to +60 mV. A possible limitation in the interpretation of data obtained in the studies just cited is that in none of these reports was the presence of an oscillation in intracellular Ca^{2+} shown (imaged directly or inferred by recording tension). Arlock and Katzung (2), Noble (65), and Brown et al. (7) were able to fit experimental data to mathematical models containing electrogenic Na-Ca exchange, which supported their conclusions that the Na-Ca exchange mechanism was the dominant charge carrier for i_{TI}. Lipp and Pott (55) recorded a spontaneous transient inward current in single dialyzed cultured guinea pig ventricular myocytes. This current was accompanied by a strong contraction, and it shared many properties with i_{TI}. The current remained inward with voltage steps up to +75 mV, and its characteristics were most compatible with a Na-Ca exchanger transporting 3 Na^+ to 1 Ca^{2+}. One unusual feature of the current was that its activation did not require Ca^{2+} overload. Lipp and Pott concluded that electrogenic Na-Ca exchange was the dominant charge carrier of the spontaneous transient inward current they studied. They also suggested that Na-Ca exchange might participate in establishing conditions [i.e., raising intracellular Ca (Ca_i)] needed to initiate transient inward current, and that Ca^{2+} release from the SR could occur in the absence of massive cellular Ca^{2+} overload. A recent observation in atrial coronary sinus cells has been used to argue further in favor of a Na-Ca exchange mechanism (78; see also ref. 2). A fast-flow chamber permitted the rapid replacement of Na^+ with Li^+, which resulted in the disappearance of delayed afterdepolarizations. Li^+ has been reported to substitute nearly equally for Na^+ in single channel recordings of Ca^{2+}-activated nonselective cation channels (21, 81). Tseng and Wit (78) argued that the disappearance of delayed afterdepolarizations was not expected for a Li^+-permeable nonselective cation channel mechanism, and they interpreted their findings to be consistent with a Na-Ca exchange-mediated mechanism in which Li^+ can not substitute for Na^+.

The experimental approach of searching for reversal potentials has produced conflicting results. In part, this could arise from other coexisting membrane currents and from tissue differences. Therefore, other experimental approaches to the mechanism for i_{TI} must be sought, and recent experimental observations have provided further insights. The opening (or closing) of a membrane channel should be accompanied by a change in membrane conductance, whereas the movement of charge on an exchange pump should not be associated with a conductance change. In embryonic heart cell aggregates exposed to caffeine (13), which induces a transient inward current resembling i_{TI}, no membrane conductance changes were seen during the inward current. A similar brief report has appeared for neonatal rat cardiac cells exposed to ouabain (1×10^{-4} M) or K^+-free medium (80). The interpretation of these results requires some caution, however, since small conductance changes may not be readily apparent. A decrease in membrane conductance during i_{TI} was reported in Purkinje fibers made toxic with strophanthidin (54). The experimental records suggest that a decrease in the conductance was already present at the onset of i_{TI}, possibly reflecting activation of an additional membrane current. Recently, Mechmann and Pott (61) observed i_{TI} in single cultured guinea pig atrial myocytes. Its induction was associated with depolarization or with exposure to caffeine. The current depended on membrane potential and on the transmembrane gradients for Na^+ and Ca^{2+} in a manner expected for electrogenic Na-Ca exchange. They also saw single channel currents that were associated with presumptive increase in intracellular Ca^{2+} (see also refs. 45 and 55).

Another approach taken by Cannell and Lederer (10) was to attempt to disable the Na-Ca exchange mechanism by removal of Na^+, while leaving the Ca^{2+}-activated nonselective cation channel mechanism intact. They reasoned that, if i_{TI} were the result of the Na-Ca exchange mechanism, then it should be unavailable, whereas if i_{TI} were the result of a nonselective cation channel, it still should be detected. They used Na^+-free isotonic Ca^{2+} Tyrode's solution to inhibit Na-Ca exchange, and with these conditions they were able to record aftercontrac-

tions and an oscillatory current resembling i_{TI} with a reversal potential near −40 mV. Power spectral analysis of the current and tension records showed similar frequency contents, and a dependence of current amplitude on the tension amplitude was shown. Because their findings were obtained in a Na^+-free environment, they concluded that inward Na-Ca exchange current could not be present. Rather, the persistence of the transient inward current constituted a powerful argument in favor of a Ca^{2+}-activated membrane channel mechanism for i_{TI}, with Ca^{2+} carrying the depolarizing current under these conditions. These data seem to provide the most direct test of the mechanisms proposed for i_{TI}. An unresolved question is why attempts to inhibit the Na-Ca exchange mechanism with sodium substitutes other than Ca^{2+} (Li^+, TRIS, choline, sucrose, and TMA) (see refs. 10, 36, and 40) produce i_{TI} only transiently, before it then disappears under steady-state conditions. One possibility is that the Ca^{2+} overload adequate to sustain i_{TI} cannot be maintained unless the external Ca^{2+} concentration is very high. Cannell and Lederer (10) pointed out that, while their data support a Ca^{2+}-activated nonselective cation channel as the major mechanism for i_{TI}, their results did not exclude Na-Ca exchange from contributing to delayed afterdepolarizations. They also suggested that i_{TI} might be activated by the transient rise in intracellular Ca^{2+} that occurs during normal action potentials and, if so, it would contribute to the plateau phase of the cardiac action potential. Recently, Kimura (45) has reported the presence of an i_{TI}-like current in Ca^{2+}-loaded isolated guinea pig ventricular cells. The current was inward at both negative and positive voltages. Block of the Na-Ca exchanger by the replacement of Na^+ with Li^+ reduced the current amplitude (but failed to abolish it), and it reversed polarity near 0 mV. These preliminary findings were interpreted to suggest the presence of both Na-Ca exchange current and a Ca^{2+}-activated nonspecific cation channel current.

In summary, there are two hypotheses to explain delayed afterdepolarizations or i_{TI}, both dependent on a process sensitive to intracellular Ca^{2+}. A nonselective sarcolemmal cation channel that is activated by Ca^{2+} has been identified, and it would be sufficient to explain the membrane phenomena. Alternatively, increased intracellular Ca^{2+} will activate Na-Ca exchange to increase Ca^{2+} efflux and Na^+ influx; and this results in an inward, depolarizing exchange current. Persuasive evidence exists for both mechanisms, and it seems increasingly likely that both could be involved. The sensitivity for each mechanism to a rise in Ca^{2+}, however, may be different with small rises in Ca^{2+} activating only Na-Ca exchange, whereas with larger rises in Ca^{2+}, both the Na-Ca exchange and the Ca^{2+}-activated nonselective cation channel mechanisms may operate. Determination of the quantitative contribution of each mechanism in the various experimental models used will require development of specific blockers of the nonspecific cation channel and the Na-Ca exchange system and further study of their separate dependencies on Ca^{2+}.

VI. Early Afterdepolarizations—Is the Mechanism the Same?

Early afterdepolarizations are secondary depolarizations that occur before complete repolarization of the cardiac action potential. They are another potentially arrhythmogenic mechanism. The basis for early afterdepolarizations is poorly understood, and several cellular processes have been implicated in their generation (for reviews, see refs. 37 and 86). It also has been shown recently that early afterdepolarizations may be initiated from more than one range of voltages (17) which may suggest more than one mechanism. One postulated mechanism is that both early and delayed afterdepolarizations may have a common basis in intracellular Ca^{2+} oscillations resulting from Ca^{2+} overload of the SR (11). Perhaps the strongest evidence supporting a common role for intracellular Ca^{2+} oscillations is that "aftercontractions" can be recorded with early as well as delayed afterdepolarizations (37). Furthermore, both early and delayed afterdepolarizations are forms of triggered activity and require an initiating event, such as one or more action potentials. Both early and delayed afterdepolarizations can be suppressed by a number of drugs, including Ca^{2+} channel-blocking drugs. However, several lines of evidence suggest that early and delayed afterdepolarizations may not share the same cellular mechanism. (*a*) Early afterdepolarizations, unlike delayed afterdepolarizations, are increasingly likely to occur at low stimulation frequencies and commonly are associated with prolongation of the cardiac action potential. (*b*) When constant current pulses are used to polarize the cell membrane to different initiating voltages, the resulting early or delayed afterdepolarizations reach different peak voltages. For early afterdepolarizations, this relationship has a steep inverse slope (37), whereas for delayed afterdepolarizations, the slope of the relationship is in the opposite direction (82). (*c*) The peak voltage of early afterdepolarizations in Purkinje fibers may exceed the reversal potential reported for i_{TI} (37). (*d*) Interventions that modify intracellular Ca^{2+} (e.g., BAPTA, ryanodine) suppress delayed but not early afterdepolarizations (59). (*e*) i_{TI} and its aftercontraction can be induced separately in tissue already having early afterdepolarizations (36). (*f*) Early afterdepolarizations arising at action potential plateau voltages have been shown to depend on the availability of L-type Ca^{2+} current (36). One mechanism postulated to explain early afterdepolarizations is that their induction requires lengthening of the action potential plateau within a voltage range where L-type Ca^{2+} channels can recover from inactivated to closed states, and then reopen. Thus with repolarization, recovery of depolarizing current could occur through the L-type Ca^{2+} "window" current and initiate early afterdepolarizations

(35–37). This mechanism best describes early afterdepolarizations initiated at action potential plateau voltages, and it does not require Ca^{2+} overload of the cardiac cell. A role may still exist for intracellular Ca^{2+}, however, since it modulates the transmembrane Ca^{2+} ion gradient, and together with voltage it regulates the inactivation kinetics of Ca^{2+} channels.

VII. Relationship of Ca^{2+} and Delayed Afterdepolarizations to Clinical Arrhythmias

It has long been recognized by physicians that toxic concentrations of cardiac glycosides cause arrhythmias that sometimes can be fatal (for review see ref. 75). Using extracellular recording techniques in isolated tissue preparations, early investigators (6) introduced the idea that an oscillatory electrical event was triggered by the action potential and might cause arrhythmias. Subsequent studies showed that toxic concentrations of cardiac glycosides led to altered ventricular excitability (for example, see ref. 56) and caused an overdrive-dependent acceleration in ventricular pacemaker rate (for example, see ref. 87). This set the stage for the present method of provoking delayed afterdepolarizations in isolated tissue by using rapid pacing combined with exposure of the tissue to high levels of cardiotonic steroids, catecholamines, or other interventions that promote Ca^{2+} overload. The ability to generate delayed afterdepolarizations under laboratory conditions that resembled clinical states supported the idea that delayed afterdepolarizations could be an important mechanism underlying some cardiac arrhythmias.

Evidence supporting a role for delayed afterdepolarizations in myocardial ischemia can be found. Triggered rhythms have been reported in association with delayed afterdepolarizations in canine endocardial tissue obtained from 1-day-old myocardial infarctions (24). Enhancement of the delayed afterdepolarizations with low concentrations of cardiac glycosides has been suggested as a basis for the potentially deleterious effects of cardiac glycosides in acute myocardial infarction (32). Studies in chronically and recently infarcted tissue using ion-selective microelectrodes (20, 47) have shown depolarization of the cell membrane and elevation of intracellular Na^+, both of which could promote Ca^{2+} loading of the cells. In isolated tissues, both induction and suppression of delayed afterdepolarizations have been observed in experimental ischemia-reperfusion models (14, 29, 33, 57), and evidence exists supporting Ca^{2+} overload as the underlying mechanism. Finally, electrophysiologically active toxic ischemic metabolites (i.e., lysophosphatidylcholine, etc.) have been reported to provoke afterdepolarizations and triggered activity in isolated cardiac tissue (3, 68).

A role for Ca^{2+} overload and Ca^{2+}-dependent ionic currents in both the *initiation* and *maintenance* of ventricular fibrillation has been proposed by Clusin and coworkers (12). These authors suggested that Ca^{2+} overload of cells could initiate ventricular fibrillation and that its maintenance was mediated by the same cellular process that gave rise to the initiation of afterdepolarizations and abnormal automaticity. This hypothesis provided a mechanism for the reported beneficial effects of Ca^{2+} channel blockers in experimental ventricular fibrillation as well as for deleterious effects of β-adrenergic receptor agonists. Findings supportive of this hypothesis were reported recently by Merillat et al. (63). They provoked ventricular fibrillation in rabbit hearts loaded with Ca^{2+} by removal of $[K]_o$ or exposure to ouabain. Subsequent lowering of extracellular Ca^{2+} to 80 μM was shown to abolish the ventricular fibrillation. They concluded that Ca^{2+} overload caused ventricular fibrillation in their model, which ceased when Ca^{2+} overload was reversed. Kusuoka et al. (48) studied perfused ferret hearts loaded with Ca^{2+} by exposure to strophanthidin and also showed the development of ventricular fibrillation and an associated pressure oscillation thought to reflect contractile asynchrony. The addition of ryanodine to the perfusate rapidly eliminated the pressure oscillations, but failed to stop the ventricular fibrillation. They concluded that the ventricular fibrillation they studied was not maintained by a primary oscillation of intracellular Ca^{2+}, and they suggested that other arrhythmogenic mechanisms, such as reentry or abnormal automaticity, might sustain the arrhythmia. These results, however, did not exclude a role for mechanisms dependent on Ca^{2+} overload in the initiation of ventricular fibrillation. Comparison of these recent reports is difficult, in part because of differences between the experimental models and techniques. Further experimental insight is needed before the role of Ca^{2+} overload in the initiation and maintenance of ventricular fibrillation can be defined.

It has yet to be proved that delayed afterdepolarizations cause clinical arrhythmias. The majority of clinical evidence derives from the extrapolation of data obtained with experimental pacing protocols used to produce delayed afterdepolarizations and initiate spontaneous rhythms in isolated tissue. Certain clinical criteria have been developed that may be useful in differentiating arrhythmias initiated by delayed afterdepolarizations from those initiated by other arrhythmogenic mechanisms (58, 70, 86). The major differentiation is from reentry, since abnormal automaticity is generally not included as a triggered rhythm, and early afterdepolarizations are bradycardia dependent. The clinical criteria suggested that cardiac arrhythmias induced by delayed afterdepolarizations should include the characteristics that the rhythm is triggered and how this occurs (i.e., cardiac glycoside toxicity, catecholamines, etc.), its reproducibility and probability of enhancement with prior rapid pacing, the relationship of the escape interval to the preceding dominant cycle length or pacing frequency, and the characteristics and reproducibility of arrhythmia termination by single impulses and overdrive pacing. Although progress continues in developing clinically use-

ful criteria for defining underlying cellular mechanisms (58), the unequivocal separation of arrhythmogenic mechanisms by these clinical criteria frequently is not possible, and recent studies (38) showing that the characteristics of delayed afterdepolarizations are not the same in different parts of the heart may further confuse the interpretation of clinical criteria. At this point, studies utilizing surface and invasive electrophysiological techniques indicate that the strongest evidence for a role of delayed afterdepolarizations is in accelerated junctional rhythms in digitalis toxicity (71), and possibly in some forms of ventricula tachycardia (see refs. 8, 58, and 86).

Direct recordings from the endocardial or epicardial surfaces of the heart permit the recording of the monophasic action potential and are an additional approach to studying the role of delayed afterdepolarizations. Although the theoretical basis for these contact recordings is incompletely explained, experimental validation suggests that the scaled monophasic action potential is a reasonable approximation of the directly recorded transmembrane action potential (30, 53). Furthermore, this technique may permit the identification of afterdepolarizations in vivo (see ref. 53), and the approach of recording the monophasic action potential seems to be a promising new methodology. While valuable, these types of recordings potentially are limited by their focal nature and by complexities arising from the "field of view" of the contact electrode (see ref. 52). Another approach to the identification of underlying arrhythmogenic mechanisms in the pattern of excitability as defined by the strength-interval relationship. Intracellular and extracellular stimulation techniques have shown characteristic biphasic changes in excitability in association with delayed afterdepolarizations, and they may be a marker for their presence (73, 77). Finally, the direct imaging of intracellular Ca^{2+} in the intact heart may provide a powerful new tool.

In addition to the direct initiation of abnormal rhythms by the delayed afterdepolarization reaching threshold voltage, other possible arrhythmogenic roles exist for them. Delayed afterdepolarizations failing to reach threshold voltage themselves are associated with substantial changes in current threshold (77). It has also long been known that delayed afterdepolarizations can alter conduction velocity and may produce conduction block (67, 73), presumably as a result of a change in excitability (19). These changes could provide the conditions necessary to establish conduction block such that reentrant rhythms could be initiated. In this way the afterdepolarization would serve to initiate the tachyarrhythmia, but would not be required for its maintenance. Another possible role for delayed afterdepolarizations is that the associated time-dependent changes in diastolic excitability also could contribute to rate-dependent conduction disturbances. Clearly, the dissection of the cellular mechanisms at the clinical level is complex, and it is likely to require new drugs that are highly selective for specific mechanisms and more accurate and detailed in vivo recording techniques.

REFERENCES

1. Allen, D. G., Eisner, D. A., and Orchard, C. H.: Characterization of oscillations of intracellular calcium concentration in ferret ventricular muscle. J. Physiol. (Lond.) **352:** 113–128, 1984.
2. Arlock, P., and Katzung, B. G.: Effects of sodium substitutes on transient inward current and tension in guinea-pig and ferret papillary muscle. J. Physiol. (Lond.) **360:** 105–120, 1985.
3. Arnsdorf, M. F., and Sawicki, G. J.: The effects of lysophosphatidylcholine, a toxic metabolite of ischemia, on the components of cardiac excitability in sheep Purkinje fibers. Circ. Res. **49:** 16–30, 1981.
4. Barcenas-Ruiz, L., and Wier, W. G.: Voltage dependence of intracellular $[Ca^{2+}]_i$ transients in guinea pig ventricular myocytes. Circ. Res. **61:** 148–154, 1987.
5. Boyette, M. R., Kirby, M. S., and Orchard, C. H.: Beat-to-beat changes in calcium current (i_{Ca}) during calcium overload in mammalian ventricular myocytes (abstract). J. Physiol. (Lond.) 391:44P, 1987.
6. Bozler, E.: The initiation of impulses in cardiac muscle. Am. J. Physiol. **138:** 273–282, 1943.
7. Brown, H. F., Noble, D., Noble, S. J., and Taupignon, A. I.: Relationship between the transient inward current and slow inward currents in the sino-atrial node of the rabbit. J. Physiol. (Lond.) **370:** 299–315, 1986.
8. Brugada, P., and Wellens, H. J. J.: The role of triggered activity in clinical ventricular arrhythmias. Pace **7:** 260–271, 1984.
9. Callewaert, G., Vereecke, J., and Carmeliet, E.: Existence of a calcium-dependent potassium channel in the membrane of cow cardiac Purkinje cells. Pfluggers Arch. **406:** 424–426, 1986.
10. Cannell, M. B., and Lederer, W. J.: The arrhythmogenic current i_{TI} in the absence of electrogenic sodium-calcium exchange in sheep cardiac Purkinje fibres. J. Physiol. (Lond.) **374:** 201–219, 1986.
11. Capogrossi, M., and Lakatta, E. G.: Frequency modulation and synchronization of spontaneous oscillations in cardiac cells. Am. J. Physiol. **248:** H412–H418, 1985.
12. Clusin, W. T., Bristow, M. R., Karagueuzian, H. S., Katzung, B. G., and Schroeder, J. S.: Do calcium-dependent ionic currents mediate ischemic ventricular fibrillation? Am. J. Cardiol. **49:** 606–612, 1982.
13. Clusin, W. T., Fischmeister, R., and DeHaan, R. L.: Caffine-induced current in embryonic heart cells: time course and voltage dependence. Am. J. Physiol. **245:** H528–H532, 1983.
14. Coetzee, W. A., and Opie, L.: Effects of components of ischemia and metabolic inhibition on delayed afterdepolarizations in guinea pig papillary muscle. Circ. Res. **61:** 157–165, 1987.
15. Colquhoun, D., Neher, E., Reuter, H., and Stevens, C. F.: Inward current channels activated by intercellular Ca in cultured cardiac cells. Nature (Lond.) **294:** 752–754, 1981.
16. Cranefield, P. F.: Action potentials, afterpotentials, and arrhythmias. Circ. Res. **41:** 414–423, 1977.
17. Damiano, B. P, and Rosen, M. R.: Effect of pacing on triggered activity induced by early afterdepolarizations. Circulation **69:** 1013–1025, 1984.
18. Deitmer, J. W., and Ellis, D.: The intracellular sodium activity of cardiac Purkinje fibres during inhibition and re-activation of the Na-K pump. J. Physiol. (Lond.) **284:** 241–259, 1978.
19. Dominguez, G., and Fozzard, H. A.: Influence of extracellular K^+ concentration on cable properties and excitability of sheep Purkinje fibres. Circ. Res. **26:** 565–574, 1970.
20. Dresdner, K. P., Kline, R. P., and Wit, A. L.: Intracellular K^+ activity, intracellular Na^+ activity, and maximum diastolic potential of canine subendocardial Purkinje cells from one-day-old infarcts. Cir. Res. **60:** 122–132, 1987.
21. Ehara, T., Noma, A., and Ono, K.: Calcium-activated nonselective cation channel in ventricular cells isolated from adult guinea-pig hearts. J. Physiol. (Lond.) **403:** 117–133, 1988.
22. Eisner, D. A., and Lederer, W. J.: Na-Ca exchange: stoichiometry and electrogenicity. Am. J. Physiol. **248:** C189–C202, 1985.
23. Eisner, D. A., and Valdeolmillos, M.: A study of intracellular calcium oscillations in sheep cardiac Purkinje fibres measured at the single cell level. J. Physiol. (Lond.) **372:** 539–556, 1986.
24. El-Sherif, N., Gough, W. B., Zeiler, R. H., and Mehra, R.: Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. Circ. Res. **52:** 566–579, 1983.
25. Fabiato, A.: Rapid ionic modifications during the aequorin-dependent calcium transient in a skinned cardiac Purkinje cell. J. Gen. Physiol. **85:** 189–246, 1985.
26. Fabiato, A.: Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J. Gen. Physiol. **85:** 247–289, 1985.
27. Fabiato, A.: Stimulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine Purkinje cell. J. Gen. Physiol. **85:** 291–320, 1985.

28. FERRIER, G. R.: Digitalis arrhythmias, role of oscillatory afterpotentials. Prog. Cardiovasc. Dis. **19:** 459–474, 1977.
29. FERRIER, G. R., MOFFAT, M. P., AND LUKAS, A.: Possible mechanisms of ventricular arrhythmias elicited by ischemia followed by reperfusion: studies on isolated canine ventricular tissues. Circ. Res. **56:** 184–194, 1985.
30. FRANZ, M. R., BURKHOFF, D., SPURGEON, H., WEISFELT, M. L., AND LAKATTA, E. G.: In vitro validation of a new cardiac catheter technique for recording monophasic action potentials. Eur. Heart J. **7:** 34–41, 1986.
31. HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B., AND SIGWORTH, F. J.: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch. **391:** 85–100, 1981.
32. HARIMAN, R. J., ZEILER, R. H., GOUGH, W. B., AND EL-SHERIF, N.: Enhancement of triggered activity in ischemic Purkinje fibers by ouabain: a mechanism of increased susceptibility to digitalis toxicity in myocardial infarction. J. Am. Coll. Cardiol. **5:** 672–679, 1985.
33. HAYASHI, H. H., PONNAMBALAM, C., AND MCDONALD, T. F.: Arrhythmic activity in reoxygenated guinea pig papillary muscles and ventricular cells. Circ. Res. **61:** 124–133, 1987.
34. HILL, J. A., JR., CORONADO, R., AND STRAUSS, H. C.: Reconstitution and characterization of a calcium-activated channel from heart. Circ. Res. **62:** 411–415, 1988.
35. HIRANO, Y., AND JANUARY, C. T.: Direct recording of L-type Ca^{++} "window" current in cardiac Purkinje cells (abstract). Biophys. J. in press, 1989.
36. JANUARY, C. T., AND RIDDLE, J. M.: Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca^{++} current. Circ. Res. in press, 1989.
37. JANUARY, C. T., RIDDLE, J. M., AND SALATA, J. J.: A model for early afterdepolarizations: induction with the Ca^{++} channel agonist Bay k 8644. Circ. Res. **62:** 563–571, 1988.
38. JOHNSON, N., DANILO, P., JR., WIT, A. L., AND ROSEN, M. R.: Characteristics of initiation and termination of catecholamine-induced triggered activity in atrial fibers of the coronary sinus. Circulation **74:** 1168–1179, 1986.
39. KARAGUEUZIAN, H. S., AND KATZUNG, B. G.: Relative inotropic and arrhythmogenic effects of five cardiac steroids in ventricular myocardium: oscillatory afterpotentials and the role of endogenous catecholamines. J. Pharmacol. Exp. Ther. **218:** 348–356, 1981.
40. KARAGUEUSIAN, H. S., AND KATZUNG, B. G.: Voltage-clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. J. Physiol. (Lond.) **327:** 255–271, 1982.
41. KASS, R. S., LEDERER, W. J., TSIEN, R. W., AND WEINGART, R.: Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. J. Physiol. (Lond.) **281:** 187–208, 1978.
42. KASS, R. S., AND TSIEN, R. W.: Fluctuation in membrane current driven by intracellular calcium in cardiac Purkinje fibers. Biophys. J. **38:** 259–269, 1982.
43. KASS, R. S., TSIEN, R. W., AND WEINGART, R.: Ionic basis of transient inward current induced by strophanthidin in cardiac Purkinje fibres. J. Physiol. (Lond.) **281:** 209–226, 1978.
44. KATZ, A. M., TAKENAKA, H., AND WATRAS, J.: The sarcoplasmic reticulum. In The Heart and Cardiovascular System, ed. by H. A. Fozzard, H. Haber, R. B. Jennings, A. M. Katz, and H. E. Morgan, pp. 731–746, Raven Press, New York, 1986.
45. KIMURA, J.: Na-Ca exchange and Ca-sensitive non-selective cation current components of transient inward current in isolated cardiac ventricular cells of the guinea-pig (abstract). J. Physiol. (Lond.) in press, 1988.
46. KIMURA, J., SHUNICH, M., AND NOMA, A.: Identification of sodium-calcium exchange current in single ventricular cells of guinea-pig. J. Physiol. (Lond.) **384:** 199–222, 1987.
47. KIMURA, S., BASSETT, A. L., GAIDE, M. S., KOZLOVSKIS, P. L., AND MYERBURG, R. J.: Regional changes in intracellular potassium and sodium activity after healing of experimental myocardial infarction in cats. Circ. Res. **58:** 202–208, 1986.
48. KUSUOKA, H., JACOBUS, W. E., AND MARBAN, E.: Calcium oscillation in digitalis-induced ventricular fibrillation: pathogenic role and metabolic consequences in isolated ferret hearts. Circ. Res. **62:** 609–619, 1988.
49. LAKATTA, E. G., AND LAPPE, D. L.: Diastolic scattered light fluctuation, resting force, and twitch force in mammalian cardiac muscle. J. Physiol. (Lond.) **315:** 369–394, 1981.
50. LEDERER, W. J., AND TSIEN, R. W.: Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibers. J. Physiol. (Lond.) **263:** 73–100, 1976.
51. LEE, C. O., AND DAGOSTINO, M.: Effect of strophanthidin on intracellular Na ion activity and twitch tension in constantly driven canine cardiac Purkinje fibers. Biophys. J. **40:** 185–196, 1982.
52. LEVINE, J. H., KADISH, A. H., MOORE, E. N., GUARNIERI, T., AND SPEAR, J. F.: The MAP field of view: implications for recording phenomena (abstract). J. Am. Coll. Cardiol. **9:** 253A, 1987.
53. LEVINE, J. H., SPEAR, J. F., GUARNIERI, T., WEISFELDT, M. L., DE LANGEN, C. D. J., BECKER, L. C., AND MOORE, E. N.: Cesium chloride-induced long QT syndrome: demonstration of afterdepolarizations and triggered activity in vivo. Circulation **72:** 1092–1103, 1985.
54. LIN, C.-I., KATAKE, H., AND VASSALE, M.: On the mechanism underlying the oscillatory current in cardiac Purkinje fibers. J. Cardiovasc. Pharmacol. **8:** 906–914, 1986.
55. LIPP, P., AND POTT, L.: Transient inward current in guinea-pig atrial myocytes reflects a change of sodium-calcium exchange current. J. Physiol. (Lond.) **397:** 601–630, 1988.
56. LOWN, B.: Electrical stimulation to estimate the degree of digitalization. Am. J. Cardiol. **22:** 251–259, 1968.
57. LUCAS, A., AND FERRIER, G. R.: Interaction of ischemia and reperfusion with subtoxic concentrations of acetylstrophanthidin in isolated cardiac ventricular tissues: effects on mechanisms of arrhythmia. J. Mol. Cell. Cardiol. **18:** 1143–1156, 1986.
58. MALFATTO, G., ROSEN, T. S., AND ROSEN, M. R.: The response to overdrive pacing of triggered atrial and ventricular arrhythmias in the canine heart. Circulation **77:** 1139–1148, 1988.
59. MARBAN, E., ROBINSON, S. W., AND WIER, W. G.: Mechanisms of arrhythmogenic delayed and early afterdepolarizations in ferret ventricular muscle. J. Clin. Invest. **78:** 1185–1192, 1986.
60. MATSUDA, H., NOMA, A., KURACHI, Y., AND IRISAWA, H.: Transient depolarization and spontaneous fluctuations in isolated single cells from guinea pig ventricles. Calcium-mediated membrane potential fluctuations. Circ. Res. **51:** 142–151, 1982.
61. MECHMANN, S., AND POTT, L.: Identification of Na-Ca exchange current in single cardiac myocytes. Nature (Lond.) **319:** 597–599, 1986.
62. MEHDI, T., AND SACHS, F.: Voltage clamp of isolated cardiac Purkinje cells (abstract). Biophys. J. **21:** 165a. 1978.
63. MERILLAT, J. C., LAKATTA, E. G., SPURGEON, H. A., AND GUARNIERI, T.: Ionic debrillation of the calcium overloaded heart (abstract). J. Am. Coll. Cardiol. **9:** 127A, 1987.
64. MULLINS, L. J.: The generation of electric currents in cardiac fibers by Na/Ca exchange. Am. J. Physiol. **236:** C103–C110, 1979.
65. NOBLE, D.: The surprising heart: a review of recent progress in cardiac electrophysiology. J. Physiol. (Lond.) **353:** 1–50, 1984.
66. ORCHARD, C. H., EISNER, D. A., AND ALLEN, D. G.: Oscillation of intracellular Ca^{2+} in mammalian cardiac muscle. Nature (Lond.) **304:** 735–738, 1983.
67. PEON, J., FERRIER, G. R., AND MOE, G. K.: The relationship of excitability to conduction velocity in canine Purkinje tissue. Circ. Res. **43:** 125–135, 1978.
68. POGWIZD, S. M., ONUFER, J. R., KRAMER, J. B., SOBEL, B. E., AND CORR, P. B.: Induction of delayed afterdepolarizations and triggered activity in canine Purkinje fibers by lysophosphoglycerides. Circ. Res. **59:** 416–426, 1986.
69. REEVES, J.: The sarcolemmal sodium-calcium exchange system. *In* Regulation of Calcium Transport across Muscle Membranes, Current Topics in Membranes and Transport, ed. by A. Shamoo, vol. 25, pp. 77–127, Academic Press, New York, 1986.
70. ROSEN, M. R.: Is the response to programmed electrical stimulation diagnostic of mechanisms for arrhythmias? Circulation **73:** (suppl. II): II18–II27, 1980.
71. ROSEN, M. R., FISCH, C., HOFFMAN, B. R., DANILO, P., LOVELACE, D. E., AND KNOEBEL, S. B.: Can accelerated atrioventricular junctional escape rhythms be explained by delayed afterdepolarizations? Am. J. Cardiol. **45:** 1272–1284, 1980.
72. ROUSSEAU, E., SMITH, J. S., HENDERSON, J. S., AND MEISSNER, G.: Single channel and $^{45}Ca^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel. Biophys. J. **50:** 1009–1014, 1986.
73. SAUNDERS, J. H., FERRIER, G. R., AND MOE, G. K.: Conduction block associated with transient depolarizations induced by acetylstrophanthidin in isolated canine Purkinje fibers. Circ. Res. **32:** 610–617, 1973.
74. SHEU, S-S., AND FOZZARD, H. A.: Transmembrane Na^+ and Ca^{++} electrochemical gradients in cardiac muscle and their relationship to force development. J. Gen. Physiol. **80:** 325–351, 1982.
75. SMITH, T. W., ANTMAN, E. M., FRIEDMAN, P. L., BLATT, C. M., AND MARSH, J. D.: Digitalis glycosides: mechanisms and manifestations of toxicity, part III. Prog. Cardiovasc. Dis. **27:** 21–56, 1984.
76. SUTKO, J. L., AND KENYON, J. L.: Ryanodine modification of cardiac muscle responses to potassium-free solutions. Evidence for inhibition of sarcoplasmic reticulum calcium release. J. Gen. Physiol. **82:** 385–404, 1983.
77. TEREK, R. M., AND JANUARY, C. T.: Excitability and oscillatory afterpotentials in isolated sheep cardiac Purkinje fibers. Am. J. Physiol. **252:** H645–H652, 1987.
78. TSENG, G., AND WIT, A. L.: Effect of reducing $[Na^+]_o$ on catecholamine-induced delayed afterdepolarizations in atrial cells. Am. J. Physiol. **253:** H115–H125, 1987.
79. VALDEOMILLOS, M., AND EISNER, D. A.: The effects of ryanodine on calcium-overloaded sheep cardiac Purkinje fibers. Circ. Res. **56:** 452–456, 1985.
80. VAN GINNEKEN, A.: Oscillatory current in aggregates of neonatal rat heart cells (abstract). Biophys. J. **41:** 176a, 1983.
81. VASSALLE, M., AND MUGELLI, A.: An oscillatory current in sheep cardiac Purkinje fibers. Circ. Res. **48:** 618–631, 1981.
82. WASSERSTROM, J. A., AND FERRIER, G. R.: Voltage dependence of digitalis afterpontentials, aftercontractions, and inotropy. Am. J. Physiol. **241:** H646–H653, 1981.
83. WASSERSTROM, J. A., SCHWARTZ, D. J., AND FOZZARD, H. A.: Relation

between intracellular sodium and twitch tension in sheep cardiac Purkinje strands exposed to cardiac glycosides. Cir. Res. **52:** 697–705, 1983.

84. WIER, W. G., AND HESS, P.: Excitation-contraction coupling in cardiac Purkinje fibers. Effects of cardiotonic steroids on intracellular [Ca^{2+}] transient, membrane potential, and contraction. J. Gen. Physiol. **83:** 395–415, 1984.
85. WIER, W. G., KORT, A. A., STERN, M. D., LAKATTA, E. G., AND MARBAN, E.: Cellular calcium fluctuations in mammalian heart. Direct evidence from noise analysis of aequorin signals in Purkinje fibers. Proc. Natl. Acad. Sci. USA **80:** 7367–7371, 1983.
86. WIT, A. L., AND ROSEN, M. R.: Afterdepolarizations and triggered activity. *In* The Heart and Cardiovascular System, ed. by H. A. Fozzard, H. Haber, R. B. Jennings, A. M. Katz, and H. E. Morgan, pp. 1449–1490, Raven Press, New York, 1986.
87. WITTENBERG, S. M., STREULI, F., AND KLOCKE, F. J.: Acceleration of ventricular pacemakers by transient increases in heart rate in dogs during ouabain administration. Circ. Res. **26:** 705–716, 1970.
88. YELLEN, G.: Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. Nature (Lond.) **296:** 357–359, 1982.