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CELLULAR AND SUBCELLULAR MECHANISMS OF CARDIAC PACEMAKER OSCILLATIONS

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SUMMARY

Rhythmic oscillations in the membrane potential of heart cells are important in normal cardiac pacemaker activity as well as cardiac arrhythmias. Two fundamentally different mechanisms of oscillatory activity can be distinguished at the cellular and subcellular level. The first mechanism, referred to as a surface membrane oscillator, can be represented by a control loop in which membrane potential changes evoke delayed conductance changes and vice versa. Since the surface membrane potential is a key variable within the control loop, the oscillation can be interrupted at any time by holding the membrane potential constant with a voltage clamp. This mode of oscillation seems to describe spontaneous pacemaker activity in the primary cardiac pacemaker (sinoatrial node) as well as other regions (Purkinje fibre, atrial or ventricular muscle). In all tissues studied so far, the pacemaker depolarization is dominated by the slow shutting-off of an outward current, largely carried by potassium ions.

The second mechanism can be called an internal oscillator since it depends upon a subcellular rhythm generator which is largely independent from the surface membrane. Under voltage clamp, the existence of the internal oscillation is revealed by the presence of oscillations in membrane conductance or contractile force which occur even though the membrane potential is held fixed. The two oscillatory mechanisms are not mutually exclusive; the subcellular mechanism can be preferentially enhanced in any given cardiac cell by conditions which elevate intracellular calcium. Such conditions include digitalis intoxication, high Ca_o, low Na_o, low or high K_o, cooling, or rapid stimulation. Several lines of evidence suggest that the subcellular mechanism involves oscillatory variations in myoplasmic calcium, probably due to cycles of Ca uptake and release by the sarcoplasmic reticulum. The detailed nature of the Ca_i oscillator and its interaction with the surface membrane await further investigation.

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INTRODUCTION

The rhythmic beating of the heart depends upon oscillatory membrane potential changes in individual cardiac cells. The underlying mechanisms of such activity are important to understanding normal pacemaker function as well as abnormal cardiac rhythms. In this paper we will briefly discuss present views about the cellular and subcellular basis of pacemaker activity in the heart. Our main purpose is to distinguish between two fundamentally different kinds of oscillatory mechanism which co-exist in various cardiac preparations, and which come into play under various conditions. The first and most familiar type of oscillation is governed by interactions between surface membrane potential and surface membrane ion permeability. The second kind of oscillation involves a subcellular rhythm generator which drives surface membrane permeability and membrane potential from within the cell. This mechanism was put forward over thirty years ago, but has only recently received direct experimental support.

SURFACE MEMBRANE OSCILLATOR

Fig. 1 describes the first type of mechanism, which we shall refer to as a surface membrane oscillator. In this generalized scheme, membrane depolarization leads, either directly or indirectly, to a delayed conductance change which promotes outward (repolarizing) ionic current. Repolarization ensues, but this in turn causes the reverse of the earlier conductance change, thus promoting a net inward (depolarizing) ionic current. This leads to a new depolarization, and so the cycle repeats itself. The overall period of the oscillatory cycle is controlled by the delays in the individual steps. The system can be characterized by describing the individual steps (opening or closing of ion channels, charging of membrane capacity) in mathematical terms, and solving the resulting differential equation. Analytical and numerical treatments of this type of oscillation have been recently reviewed (Jack, Noble & Tsien, 1975).

Membrane potential is an essential variable in the control loop represented in Fig. 1. Most descriptions of repetitive activity in excitable tissues fall within this broad classification. One of the oldest examples is the valve relaxation oscillator (van der Pol, 1926; van der Pol & van der Mark, 1928), which may have been the first explicit model for the cardiac pacemaker. This model was proposed without the benefit of information about membrane mechanisms. More recent versions of the surface membrane oscillator are empirical models based on the analysis of membrane currents using the voltage clamp technique. Descriptions of repetitive activity are probably most advanced in nerve, where voltage-clamp techniques have been available for the longest time. So far, the general class of oscillatory mechanisms described in Fig. 1 seems to encompass results from a variety of neurophysiological preparations including squid axon (Huxley, 1959), crab axon (Connor, 1978), frog node of Ranvier (Bergman, Nonner & Stämpfli, 1968) and molluscan nerve cell bodies (Connor & Stevens, 1971; Gorman & Thomas, 1978). These are only a few representative cases. There are important variations in detail from preparation to preparation: the dominant time delay in the pacemaker depolarization may be due to the kinetics of K channels shutting off (Connor & Stevens, 1971; Gorman & Thomas, 1978) or Na channels

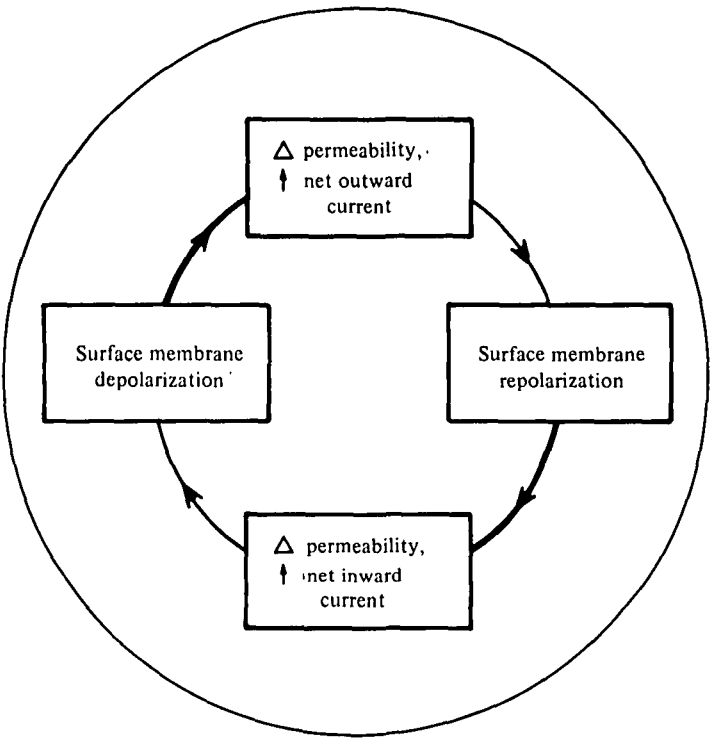

Fig. 1. Simplified representation of a surface membrane oscillator. Thin arrows indicate steps which can be interrupted by clamping the membrane potential. Thick arrows refer to potential-dependence of delayed conductance changes in the surface membrane. The mechanism of such voltage-dependence is left unspecified, but it may include Hodgkin–Huxley type gating, or an indirect gating involving calcium ions or some other intracellular messenger.

turning on (Bergman *et al.* 1968): the opening and closing of ion channels may be directly gated by membrane potential (Hodgkin & Huxley, 1952; Beginisich & Lynch, 1974) or indirectly mediated by rises and falls in intracellular calcium (Meech, 1978; Gorman & Thomas, 1978). Despite these differences, most, if not all neural preparations share the common feature that changes in membrane potential are essential to the oscillatory mechanism.

INTERNAL OSCILLATOR

More than three decades ago, Bozler (1943) proposed a fundamentally different explanation for oscillatory activity. Working with turtle ventricular muscle exposed to calcium-rich, sodium-poor solutions, he observed small, non-conducted potential changes of an oscillatory nature, accompanied by small variations in isometric force ('tonus'). One of his experiments is illustrated in Fig. 2. The electrical and mechanical oscillations follow an evoked action potential, and show a rather clear temporal correlation. Noting that the oscillations have a period of ~ 1 s, Bozler argued that they are qualitatively different from the much more rapid potential oscillations seen in nerve (Cole, 1941). As he states in the summary of the 1943 paper (p. 480), 'The

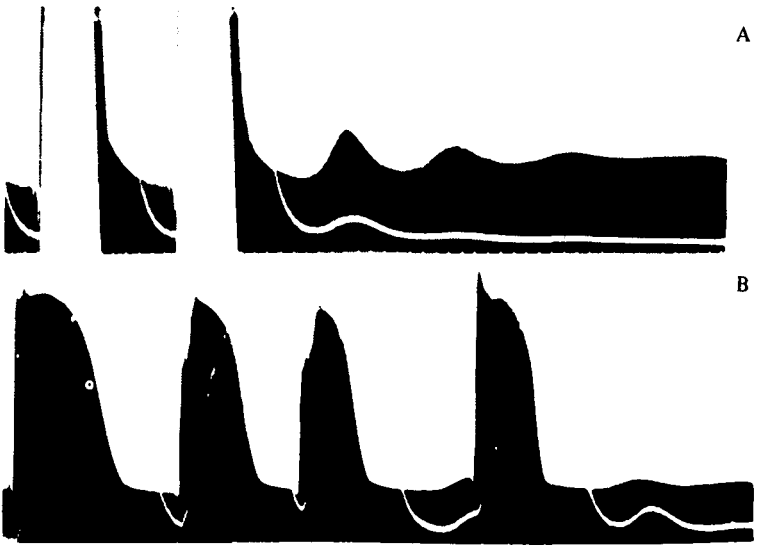

Fig. 2. Oscillatory afterpotentials and 'tonus changes' recorded simultaneously from a strip of turtle ventricular muscle. Upper curve is electrical record, lower curve is isometric force. All-or-none responses were elicited by external shocks except the last response in B, which was initiated by a local potential and was preceded by a tonus change. Time marks in A indicate intervals of 200 ms. From Bozler (1943).

tonus changes and the local potentials are probably manifestations of a more fundamental process, a fluctuation in resting metabolism. The mechanical changes are weak and hardly play any role as such. Their chief interest lies in their relation to the automaticity and rhythmicity of the muscle. It may be assumed that an increase in metabolism causes a rise in tonus and a decreased surface polarization. The decrease in polarization in turn may be considered as the last link in the chain of processes leading to the discharge of an impulse.'

Bozler's interpretation of the oscillations in turtle myocardium falls outside the framework given in Fig. 1. His views call for another kind of model, along the lines of the scheme in Fig. 3. Here, the oscillatory control loop is contained within the cell; the nature of the 'subcellular oscillator' is left unspecified but the control loop explicitly excludes the surface membrane potential. When oscillations in surface membrane permeability or surface membrane potential occur, they are seen as secondary consequences of the oscillatory mechanism.

Over the years since Bozler's experiments, explanations of cardiac pacemaker activity have generally overlooked his proposal of a subcellular, chemical oscillator. This neglect may be due in part to the apparent success of the surface membrane model (Fig. 1) in accounting for repetitive activity in other excitable tissues like nerve or skeletal muscle. Recently, the idea of an internal chemical oscillator has been revived by Rapp & Berridge (1977). These authors drew on analogies between oscillatory activity in a wide variety of cells, and proposed a modern version of the scheme in Fig. 3 as a general mechanism for cardiac pacemaker activity. Their reasoning was as follows (p. 517): 'The pacemaker potential can exist independently of action potential activity. Since both calcium and cyclic AMP can effect membrane permeability and hence membrane potential, it is possible that pacemaker waves may

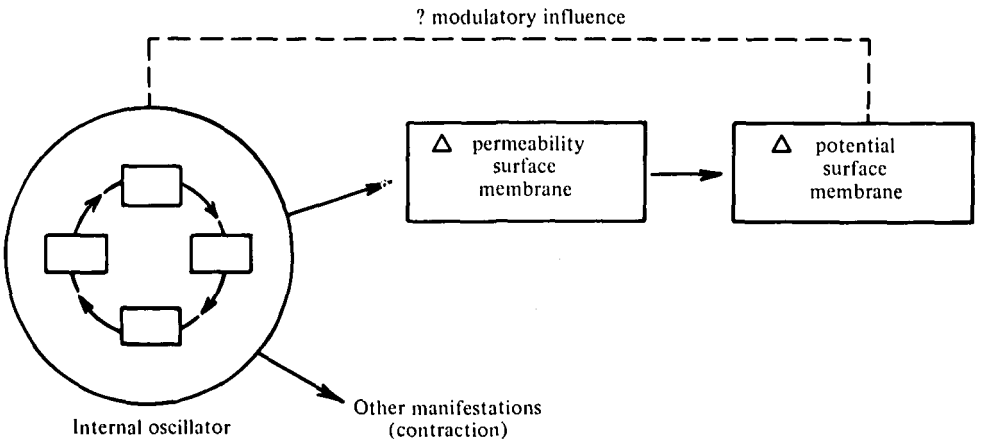

Fig. 3. Simplified view of subcellular oscillator. Basic rhythm is generated within the cell by the 'internal oscillator', which governs the surface membrane permeability and other measurable properties such as contractile force. The dashed line indicates the possibility of indirect modulation of oscillatory frequency or amplitude by the surface membrane potential.

be driven by oscillations in these messengers.' In a sense, the oscillations in calcium and cyclic AMP proposed by Rapp & Berridge are a specific example of what Bozler called 'a fluctuation in resting metabolism'.

APPLICABILITY OF OSCILLATOR MODELS

How do the proposed mechanisms fit with experimental evidence? The applicability of the surface membrane oscillator and the internal oscillator has been clarified by recent work in at least three areas: firstly, voltage-clamp analysis of activity in the natural pacemaker of the heart, the sinus venosus of frog (Brown, Giles & Noble, 1976) or the sino-atrial node of the rabbit (Noma & Irisawa, 1976); secondly, voltage-clamp experiments on the type of electromechanical oscillation first observed by Bozler (Kass *et al.* 1978); and thirdly, observations of force oscillations in cardiac preparations where the surface membrane has been 'skinned' by mechanical or chemical means (Fabiato & Fabiato, 1972; Müller, 1976; Endo & Kitizawa, 1978). Much of this work has been recently reviewed (Brown, Giles & Noble, 1977; Irisawa, 1978; Tsien, Kass & Weingart, 1978; Tsien, Weingart & Kass, 1978; Tsien & Carpenter, 1978; Fabiato & Fabiato, 1977). We will therefore restrict ourselves to a brief summary of the main points.

(1) The different types of oscillatory mechanism are not mutually exclusive. In fact, recent experiments indicate that both kinds of oscillation can occur in the same cardiac preparation, with either the surface membrane or internal oscillator mechanism dominating under different experimental conditions (see for example, Lederer & Tsien (1976), fig. 1).

(2) Independent of oscillatory mechanism, cardiac pacemaker activity can be divided into two broad categories on purely descriptive grounds (Ferrier, 1977). A variety of criteria distinguish 'oscillatory afterpotentials' from 'normal' pacemaker activity (phase 4 depolarization).

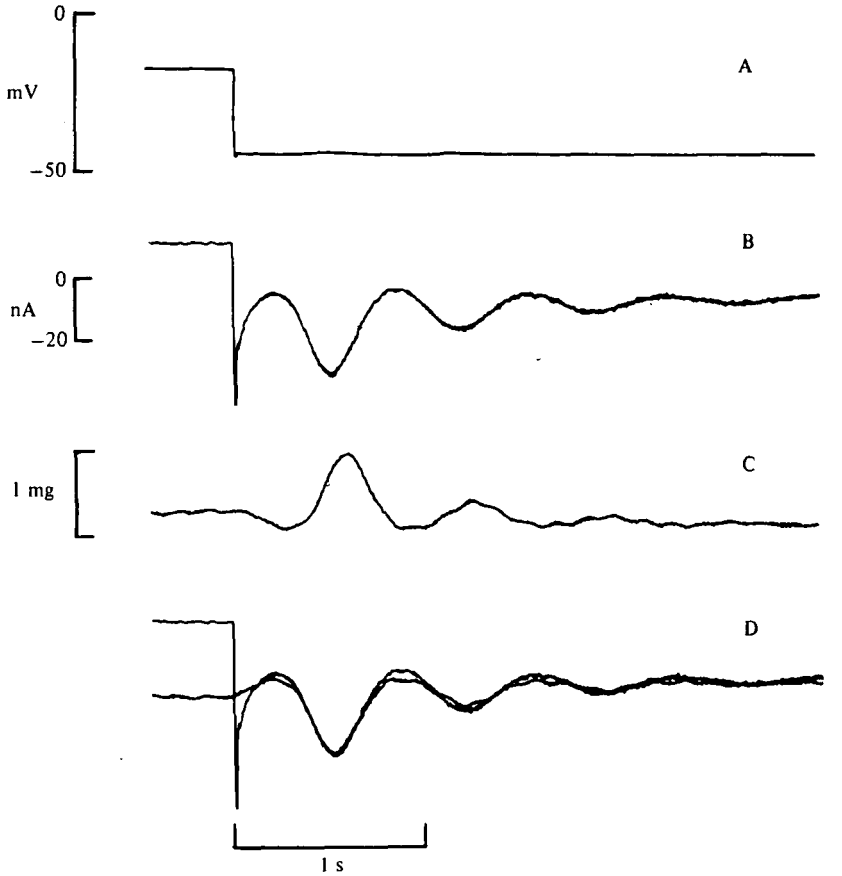

Fig. 4. Oscillatory inward currents and aftercontractions in a calf cardiac Purkinje fibre intoxicated with 1 μM strophanthidin. A repolarizing step to −44 mV (A) terminated a 10 s depolarizing pulse to −17 mV. The repolarizing step evoked a series of inward current transients (B) which were associated with oscillatory aftercontractions (C). Panel D shows the temporal relationship between current and force oscillations, the force record in C being inverted and advanced by 80 ms. From Kass *et al.* (1978).

(3) Oscillatory afterpotentials and aftercontractions are preferentially enhanced by a variety of procedures, including high Ca_o, low Na_o, low K_o, cooling, or exposure to toxic concentrations of cardiac glycoside or aglycone. All of these interventions share the capability of elevating the myoplasmic Ca activity (Kass *et al.* 1978). Unlike the normal pacemaker depolarization, oscillatory afterpotentials and aftercontractions are dramatically enhanced in the wake of a closely spaced train of action potentials.

(4) Normal pacemaker activity refers to spontaneous firing in natural pacemaker tissue (sinus node, atrio-ventricular node, or Purkinje fibre) or repetitive activity evoked by steady depolarizing current in working myocardial preparations. The ionic basis of pacemaker depolarization has been analyzed by voltage-clamp experiments (for review, see Noble, 1975; Irisawa, 1978), with a procedure first used by Vassalle (1966). Table 1 summarizes the conclusions of several investigations in different preparations. In all cases studied so far, including sinus pacemaker preparations, the pacemaker depolarization is generated by a smooth decay of outward current carried

Table 1. *Mechanism of normal pacemaker activity*

Preparation	Voltage range spanned by slow depolarization	Dominant pacemaker current	Reference
Sheep Purkinje fibre	−90 to −60	I_{K_2}	Vassalle (1966), Noble & Tsien (1968)
Sheep Purkinje fibre	−60 to −30	I_x	Hauswirth *et al.* (1969)
Frog atrial muscle	−60 to −30	I_x	Brown *et al.* (1972)
Frog sinus venosus	−60 to −30	I_x	Brown *et al.* (1976)
Rabbit S-A node	−70 to −50	I_p	Noma & Irisawa (1976)
Cat and guinea-pig ventricular muscle	−70 to −50	I_x	Katzung & Morgenstern (1977)

All currents are predominantly carried by potassium ions. I_{K_2} has a steady-state activation curve ranging from full deactivation at −90 mV to full activation at −60 mV in the absence of chronotropic drugs. All other currents show voltage-dependent activation over the range between −50 and 0 mV.

by channels which are largely, if not completely specific to potassium ions. The genesis of the pacemaker depolarization in frog sinus tissue and its modulation by neurotransmitters is reviewed in the paper by Brown, DiFrancesco & Noble (see this volume).

(5) Oscillatory afterpotentials (OAP), aftercontractions (AC) and related electromechanical fluctuations have been observed in a wide variety of preparations from different regions of the heart (Table 2). Their present-day explanation comes remarkably close to Bozler's original idea of an internal chemical oscillator. However, the evidence for this view has been obtained by voltage clamping or skinning cardiac preparations, techniques unavailable in 1943. In fact, the oscillations Bozler observed could actually have been accommodated within the framework of a surface membrane oscillator. The relatively slow time course of the oscillations does not exclude the surface oscillator mechanism, and the force oscillations could be accounted for by supposing that contractile activation was under direct control by surface membrane potential (Kaufmann, Fleckenstein & Antoni, 1963; Akselrod *et al.* 1977).

(6) The voltage-clamp technique allows surface membrane potential to be held fixed, and this provides a direct means for finding out whether the surface membrane potential lies within the oscillatory control loop (Fig. 1) or outside it (Fig. 3). As Fig. 4 illustrates, such experiments show that oscillations in force and membrane current can still be recorded at constant membrane potential. The oscillations in this example damp out following the imposed voltage step. However, small oscillatory fluctuations in force and current can be observed when the membrane potential is held constant over prolonged periods (Kass, Lederer & Tsien, 1976; Irisawa, & Noma, 1977; Mehdi & Sachs, 1978).

(7) Another argument for the existence of an internal oscillator comes from skinned fibre experiments where the surface membrane is mechanically stipped away (Fabiato & Fabiato, 1972, 1977) or rendered permeable to small molecules by treatment with EDTA (Müller, 1976) or saponin (Endo & Kitizawa, 1978). When the myoplasmic calcium concentration is elevated with light EGTA buffering, such preparations show cyclic force oscillations. These oscillations have been attributed to cycles of calcium uptake and release by the sarcoplasmic reticulum (Fabiato & Fabiato, 1972; Bloom, 1971), on the basis of effects of non-ionic detergents (Fabiato & Fabiato, 1975) and

Table 2. *Aftercontractions, oscillatory afterpotentials and related oscillatory phenomena in intact cardiac preparations*

(CTS, cardiotonic steroids; AC, aftercontraction; OAP, oscillatory afterpotential; TI, transient inward current.)

Preparation	Intervention(s)	Oscillatory event(s)	Reference
Turtle ventricular muscle	High Ca_o, low Na_o,	AC/OAP	Bozler (1943)
	high Ca_o, stretch	AC/OAP	Bozler & Delahayes (1973
Guinea-pig papillary	Cooling, high Ca_o, low Na_o, CTS	AC but no OAP	Reiter (1962, 1963)
Guinea-pig papillary, Guinea-pig atrial	Cooling, high Ca_o, low Na_o, CTS	AC/OAP	Kaufmann *et al.* (1963)
Guinea-pig atrial	Cooling, high Ca_o, rapid drive	AC	Braveny *et al.* (1966)
Guinea-pig atrial	Cooling, high Ca_o, rapid drive	AC/OAP	Jensen & Katzung (1968)
Chick cultured myocyte monolayer	High K_o	Fluctuations in movement, ± voltage changes	Pappano & Sperelakis (1969)
Cat papillary	Low Na_o, high K_o	AC/no clear OAP	Mascher (1971)
Cat papillary	High Ca_o, low Na_o epinephrine	AC/OAP	Ryo (1971)
Cow ventricular	Low Na_o, caffeine	AC/no clear OAP	Verdonck *et al.* (1972)
Guinea-pig atrial	High Ca_o, low Na_o	AC/fluctuations in force	Glitsch & Pott (1975)
Dog ventricular	Isoproterenol	AC/OAP	Nathan & Beeler (1975)
Dog Purkinje fibre	CTS, stretch	AC/OAP	Ferrier (1976)
Dog ventricular			
Mouse or chick myocytes singly or in clusters	High Ca_o, low K_o, CTS	Fluctuations in movement, voltage	Goshima (1976, 1977)
Carp atrial	No overt intervention	Fluctuations in movement, voltage	Akselrod *et al.* (1977)
Calf Purkinje fibre	CTS	AC/OAP/TI fluctuations in force, current	Lederer & Tsien (1976), Kass *et al.* (1976)
Dog Purkinje fibre, isolated cells	No overt intervention	Fluctuations in movement, current	Mehdi & Sachs (1978)
Sheep Purkinje fibre	Low K_o	AC/OAP/TI fluctuations in force, current	DiFrancesco *et al.* (1978)
Rabbit sinus node	Cooling, high Ca_o, low Na_o, high K_o	Fluctuations in movement, current	Irisawa & Noma (1977)

pharmacological agents such as caffeine, ruthenium red, azide and oligomycin (Bloom, Brady & Langer, 1974; Fabiato & Fabiato, 1975). The possibility of associated oscillations in cyclic AMP (cf. Rapp & Berridge, 1977) has not been investigated in the skinned fibres.

(8) Several lines of evidence suggest that similar oscillations in Ca_i underly the oscillations in membrane current and force seen in intact cells. Firstly, the conditions which promote the oscillatory afterpotential and aftercontraction are known to elevate myoplasmic Ca, and thus parallel the conditions for producing force oscillations in skinned myocytes. Secondly, the inhibition of Ca entry by agents such as manganese or D600 reduces the amplitude and frequency of the oscillatory events (Kass *et al.* 1978). Thirdly, local anaesthetic drugs such as tetracaine, aprindine or quinidine, which are known to interfere with Ca movements across sarcoplasmic

reticulum membranes, reduce the amplitude and frequency of the electromechanical oscillations (Goshima, 1976; Tsien *et al.* 1978). Fourthly, intracellular injection of the Ca chelator EGTA abolishes spontaneous fluctuations in current and force in isolated Purkinje cells (Mehdi & Sachs, 1978) and reduces the amplitude and frequency of the current and force oscillations in intact Purkinje fibres (Siegelbaum & Tsien, unpublished data).

(9) The oscillatory afterpotential is generated by an oscillatory inward current (TI) which has a reversal potential near -5 mV in the standard Tyrode solution. The reversal potential is sensitive to sodium removal, but not to moderate variations in other ion concentrations. The ionic pathway for the inward current is not known, but two leading possibilities are the TTX-insensitive background sodium current, and the calcium–sodium exchange.

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