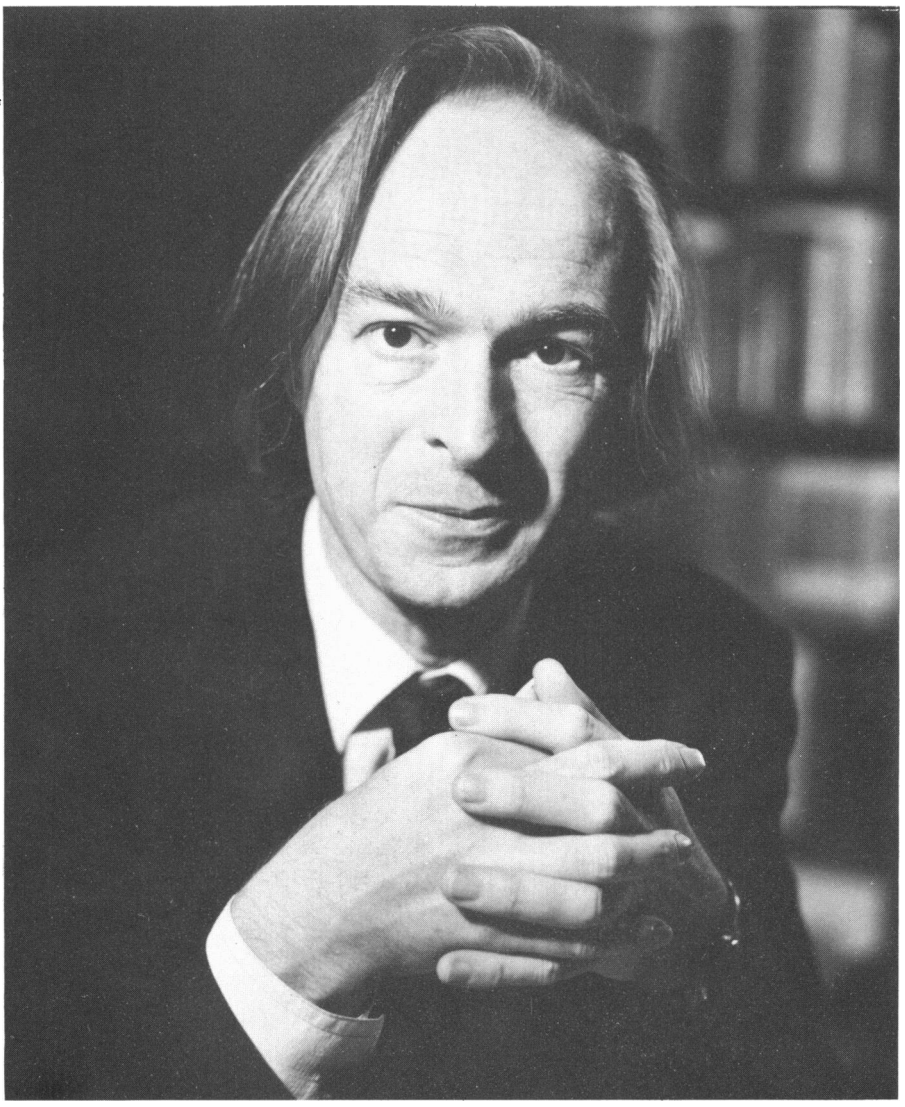

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REVIEW LECTURE*

THE SURPRISING HEART: A REVIEW OF RECENT PROGRESS IN CARDIAC ELECTROPHYSIOLOGY

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INTRODUCTION

My aim in this lecture will be to review recent progress in our understanding of cardiac pace-maker mechanisms. In doing so I shall also try to explain the major changes that have occurred recently in the interpretation of two of the important ionic current mechanisms involved: i_{K2} (the Purkinje fibre 'pace-maker' current) and i_{si} (the 'second inward' or 'slow inward' current which is known to be important in rhythmic activity in the sino-atrial (s.a.) node).

The earliest ionic current models for pace-maker activity in the heart were based on those for repetitive activity in nerve. My own first involvement in modelling the cardiac action potential and pace-maker activity (Noble, 1960, 1962*a*) was greatly influenced by the fact that Huxley (1959) had just shown that the Hodgkin–Huxley (1952) equations could reproduce the repetitive firing observed in nerve axons in Ca-deficient solutions. The mechanism involved in the 1962 model was one of those proposed by Weidmann (1951) on the basis of his resistance measurements during the cardiac pace-maker potential: that a delayed increase in K conductance, g_K, that may occur during the action potential slowly decays after repolarization so allowing any background permeability, e.g. to Na ions, to drive the membrane potential away from the K equilibrium potential, E_K. I will call this hypothesis the g_K decay hypothesis.

It is worth noting that in 1960 the evidence for such a delayed K conductance change in the heart was not too strong. Hall, Hutter & Noble (1963) described a delayed K conductance change (which they called g_{K2}) superimposed on a background K current showing inward rectification (which they called g_{K1}). But the date of that paper (coming *after* the 1962 model paper) is significant: we were partly convinced that it was worth looking for the delayed K current by the fact that the modelling work seemed to require it. I spent some considerable time convincing myself that a Na conductance plus a K ion inward rectifier was theoretically not sufficient. Experimentally though, the delayed K change was not always easy to find. We now know some of the reasons why: in many cases it is obscured by a large transient outward current, i_{to} (see below).

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Moreover, in some Purkinje fibres (as in some ventricular preparations) the delayed K change is in fact small or even negligible. It was later found to be much more prominent in atrial and nodal tissues. This was a case therefore of theory leading experiment. We were also not to know at that time that the delayed current change *during* depolarization in Purkinje fibres is not generated by the same mechanism that generates the greater part of the current change during the pace-maker depolarization *following* repolarization, nor that Na-deficient solutions would eliminate the latter.

The changes that have occurred since that early work may conveniently be divided into two main phases. First, there was a period, following the introduction of the voltage clamp to the heart by Deck, Kern & Trautwein (1964), of increasing complexity within an existing framework of interpretation. During this period, more conductance mechanisms were found but the basis for analysis of rhythmic activity remained the g_K decay hypothesis in one form or another. During this period (roughly from 1964 to 1977) the second inward (Ca) current was recorded, the delayed current changes were separated into several components and a transient outward current was analysed.

The second period, roughly from 1978 to the present time, is rather more revolutionary. Currents (such as those generated by ion pumps or ion exchange mechanisms) that were neglected in earlier work have now assumed a considerably greater importance and some existing currents, i_{K2} and i_{si} in particular, have been substantially reinterpreted. Moreover, the introduction of single-cell- and patch-clamp methods has revolutionized the techniques employed and led to the discovery of even more components of ionic current, such as the non-specific current channel described by Colquhoun, Neher, Reuter & Stevens (1981) and the components of the second inward current that I shall describe later in this review.

The result is a bewildering array of ionic current mechanisms that naturally provokes the question whether one really does need that many (for an interesting theoretical essay in doing without most of them, see Johnson, Chapman & Kootsey; 1980). There is also a sense of confusion concerning the theory of the field for, if what was by all the standard criteria firmly established as a specific K ion current can turn out to be carried largely by Na ions then it becomes important both to tighten up the theory (by taking account of previously neglected processes) and, in the light of this, to carefully reassess our conclusions concerning other ionic identities and reversal potentials. Finally, there is an urgent need to reassess the pace-maker mechanisms in the heart not only because the g_K decay hypothesis no longer applies to normal Purkinje fibre rhythm but also because, for the other main pace-maker mechanism, the s.a. node, different groups have come up recently with at least four different answers to the question – what is the main 'pace-maker' current?

I am therefore very grateful to the Physiological Society for inviting me to give this review lecture at such an opportune time and I will try to rise to the challenge: to explain the recent changes to a wider audience and to try to answer some of the questions and deal with the important issues I have just raised.

If I start with a little history that is in part to provide a perspective from which to view the impact of the new work, but also because this is a somewhat personal review lecture: the period I cover is naturally co-extensive with my own involvement and I presume that to give such an account is one of the purposes of the Physiological Society review lectures.

THE FIRST STAGE: INCREASING COMPLEXITY

The 1962 cardiac model differed from its neuronal parent in only two essential features.

(1) The leak current was separated into a background Na current, $i_{b,Na}$, and a K current mechanism, i_{K1}, showing inward-going rectification (see Hutter & Noble, 1960; Carmeliet, 1961; Hall, Hutter & Noble, 1963).

(2) The speed and magnitude of the delayed K current change (called g_{K2}) were greatly reduced compared to the relevant parameters for nerve fibres (see Hall *et al.* 1963).

Subsequent experimental work using the voltage-clamp technique led to three main developments that required additional modifications to the Hodgkin–Huxley type of model.

(i) Evidence for a role of Ca ions in generating inward current had already come from action potential experiments (Niedergerke, 1963; Orkand & Niedergerke, 1966) and flux measurements (Winegrad & Shanes 1962). In 1967, Reuter succeeded for the first time in recording a net inward current that flowed even in the absence of Na ions and which was dependent on extracellular Ca ions. The ionic current recorded in Reuter's experiment (see Fig. 1 *A*) achieved a peak inward level after about 100 ms and required several hundred milliseconds for its inactivation. This observation was important for two reasons. First, because the entry of Ca during the flow of this current could be responsible for triggering contraction and secondly, because a current with this time course would play an important role in maintaining the plateau of the action potential. The 'slow inward' or 'second inward' current has since been recorded by many workers in a wide variety of cardiac cells. However, the kinetics show a bewildering and important diversity, with inactivation time constants, for example, varying from 20 ms (see, e.g. Rougier, Vassort, Garnier, Gargouil & Coraboeuf, 1969) to more than 1 s. The importance of this diversity has, I think, been underestimated. It is of functional importance since the kinetics of the current change determine its role in the action potential mechanism. It is bewildering because, even in the same tissue (the Purkinje fibre), the variations are large. Siegelbaum & Tsien (1980) and Marban & Tsien (1982) recorded currents with much faster activation and inactivation kinetics in EGTA-injected or Cs-loaded preparations (see Fig. 1 *B* – I will comment further on the significance of this result later in this review), whereas at the other extreme Lederer & Eisner (1982) and Eisner, Lederer & Noble (1979) recorded a current that differs from those shown in Fig. 1 *A* and *B* in showing almost symmetric activation and inactivation time courses. This current (shown in Fig. 1 *C*) is best obtained over a fairly narrow range of voltages (in the region of −40 mV) and in its time course it strongly resembles another slow inward current: the transient inward current, i_{TI}, first described by Lederer & Tsien (1976) in preparations in which the Na–K exchange pump has been blocked by cardiac glycosides. An example of this kind of inward current is shown in Fig. 1 *D*. It should be noted that the voltage protocol for obtaining i_{TI} is rather different. It is usually recorded on hyperpolarizing back from a depolarizing pulse, but it can also be recorded during depolarization and I shall discuss the significance of this fact later. My reason for presenting this diverse array of 'second inward' current records together in a single Figure will also become evident later when I have reviewed more recent work;

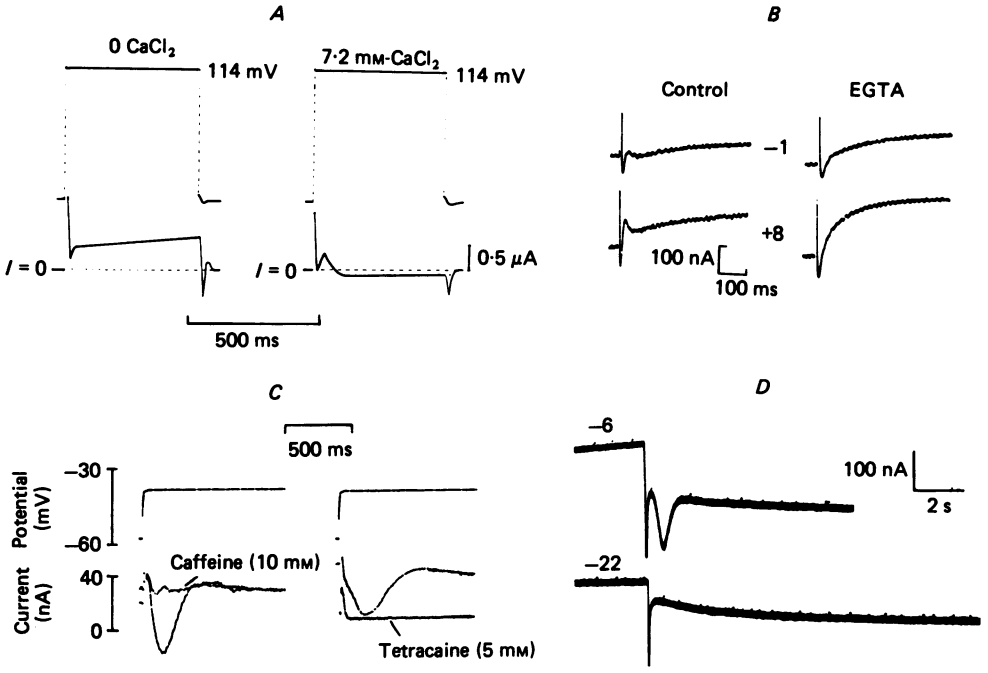

Fig. 1. Slow inward currents recorded in multicellular Purkinje fibre preparations. *A*, this shows the first slow inward current recorded by Reuter (1967). The preparation was depolarized from −80 to +34 mV in Ca-free, Na-free solution (left) and solution containing 7·2 mM-Ca ions (right). The peak inward current occurs at about 100 ms after the application of the pulse and is not fully inactivated even after 500 ms. *B*, this shows the influence of intracellular EGTA injection to remove currents (like the transient outward current) that are activated by the $[Ca]_i$ transient (Siegelbaum & Tsien, 1980). The membrane was depolarized from −46 to −1 and +8 mV. The control records on the left show the same kind of initial complex time course as in *A*. After EGTA injection, the current record becomes simpler and shows an inward current that activates and inactivates fairly rapidly. The record approaches that obtained in single cells (see Fig. 6). *C*, these records show slow inward currents with almost symmetric activation and inactivation time courses when a Purkinje fibre is clamped from −58 to −40 mV (Eisner *et al.* 1979; see also Lederer & Eisner, 1982). It is likely that this current is activated by intracellular Ca since it is abolished by caffeine (which is thought to discharge the intracellular Ca stores) and tetracaine (which abolishes the Ca release and tension response). *D*, the transient inward current, i_{TI}, recorded by Lederer & Tsien (1976) in pump-blocked Purkinje fibres. The membrane was clamped back to −72 mV following 5 s depolarizations to −6 and −22 mV in a fibre that had been exposed to 1 μM-strophanthidin. Note the similar time course of the inward currents shown in *C* and *D*, even though the voltage protocols are quite different. The thesis explored in this review is that the currents recorded in *C* and *D* may be produced by the same mechanism and that this is quite different from the mechanisms that produce the majority of the currents shown in *A* and *B*. On this view, depolarization activates two or three separate Ca or Ca-dependent inward current systems (see eqns. (2) and (3)).

(ii) In 1968 and 1969 Tsien and I (Noble & Tsien, 1968, 1969*a*,*b*) analysed the kinetics of the slow permeability changes attributed to K ions. The main outcome of that work was the discovery that, in contrast to the 1962 model, there are at least two distinct time-dependent mechanisms. The closest equivalent of g_{K2}, the delayed

K current in the 1962 work, is a current (or currents) activated in the plateau range of potentials (−40 to +20 mV). The other mechanism has a gating range at much more negative potentials in the pace-maker range (between −100 and −60 mV) and can be distinguished by its kinetic properties even when both components are present (Hauswirth, Noble & Tsien, 1972). More recently, it has been found that the two

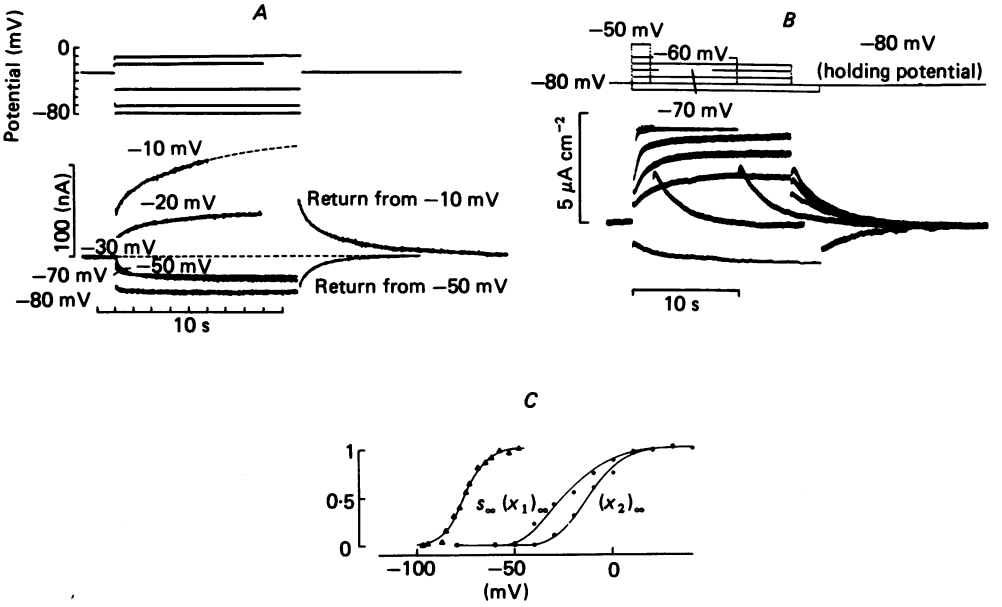

Fig. 2. Slow membrane currents in Purkinje fibres. *A*, slow membrane current recorded in the plateau range of membrane potentials by Noble & Tsien (1969). These currents have been, and still are, attributed to slow activation and deactivation of a K current. The fibre chosen for analysis here showed little or no slow current change in the pace-maker range. More usually, the plateau current needs to be separated from the pace-maker range current (see Hauswirth *et al.* 1972). *B*, slow membrane current recorded in the pace-maker range of potentials (Noble & Tsien, 1968). This was originally also attributed to K ions but is now thought to be carried by non-specific channels activated by hyperpolarization into the pace-maker range (see Fig. 3). *C*, voltage ranges of activation of these two currents as analysed by Noble & Tsien (1968, 1969). The plateau current divided into two components (labelled x_1 and x_2). The current activated in the pace-maker range (labelled s) was analysed as *deactivating* as the membrane is hyperpolarized between −60 and −90 mV. The analysis proposed by DiFrancesco (1981) is that the channels *activate* over this voltage range but, since they are presumed to carry inward rather than outward current, the net current *change* is still as shown in the records in *B*.

systems respond quite differently to divalent cations: the plateau current (which I will call i_K, but which was originally called i_x (Noble & Tsien, 1969*a*) or g_{K2} (Hall *et al.* 1963; McAllister & Noble, 1966) in previous work) is blocked by Ba ions, as is the K background current, i_{K1} (DiFrancesco, 1981*a*), whereas the 'pace-maker' current (originally called i_{K2} by Noble & Tsien (1968) is blocked by Cs ions (Isenberg, 1976; Carmeliet, 1980; DiFrancesco, 1982). Fig. 2 summarizes the kinetic properties of these currents.

(iii) The third additional current mechanism is the transient outward current, i_{to}.

This current greatly resembles the current i_A in molluscan neurones. In the case of the heart, it was first found in Purkinje fibres and is responsible, in part, for the characteristic notch in the Purkinje fibre action potential. Like the current, i_A, a large fraction of i_{to} is blocked by 4-aminopyridine, 4-AP (Kenyon & Gibbons, 1979; Boyett, 1981). There is, however, a small component (which in the calf is the only component) that remains even in the presence of 4-AP (Coraboeuf & Carmeliet, 1982). This component disappears if the transient rise in intracellular Ca ions that normally initiates contraction (in the rest of this article I will, for the sake of brevity, call this the $[Ca]_i$ transient) is abolished by injecting the Ca buffer EGTA into the cells (Siegelbaum & Tsien, 1980.) There is also a strong effect of repetitive activity on the amplitude of i_{to} (Boyett & Jewell, 1980; Boyett 1981). The early analysis of i_{to} suggested that it was a Cl current (see, e.g. Carmeliet, 1961; Dudel, Peper, Rudel & Trautwein, 1967; Fozzard & Hiraoka, 1973) and this was how it was represented in the McAllister, Noble & Tsien (1975) model (MNT). Kenyon & Gibbons (1979) however showed that its apparent Cl dependence is to a great extent due to variations in extracellular free Ca. The best evidence now suggests that it is in large part a $[Ca]_i$-activated and $[K]_o$-activated K current. This may account for the component that is blocked by 4-AP. The identity of the remaining component will be discussed later.

Thus, in addition to the four components of the 1962 model (i_{Na}, i_K, i_{K1}, and $i_{b,Na}$), these results revealed the presence of three additional currents (i_{si}, i_{K2} and i_{to}). It was for the purpose of exploring the consequences of including these additional components that McAllister, Tsien and I constructed a new model (McAllister *et al.* 1975). Being based on a wider range of experimental results, the new model had a greater explanatory range. In particular it could in principle account for the fact that Purkinje fibre rhythmic activity can occur in two different potential ranges: the normal Purkinje pace-maker range (-90 to -60 mV) and a depolarized range (-60 to -40 mV). The oscillations in the latter range are fairly similar to the natural oscillations of the s.a. node (Hauswirth, Noble & Tsien, 1969) and an early 'model' of the s.a. node rhythm was based on the view that i_{si} and i_K were the main currents involved. As we shall see later, this is close to one of the present views of s.a. node pace-maker activity: indeed I shall show that this is the only form of pace-maker activity that still conforms at all closely to the original g_K decay hypothesis.

In the MNT (McAllister *et al.* 1975) model, both kinds of pace-maker depolarization are attributable to (different) g_K decay processes. Before I start to review the more recent developments it is worth summarizing the weight of evidence that had accumulated in favour of the g_K decay hypothesis by 1975.

(a) In 1951 Weidmann performed a series of experiments that have been of seminal importance in cardiac electrophysiology. He injected small pulses of current at various times during the cardiac cycle in an attempt to measure the variations in total membrane conductance. This work confirmed the presence of all-or-nothing repolarization during the plateau (which was one of the reasons I was certain in 1960 (see also Noble, 1962*b*; Noble & Hall, 1963) that there had to be a slow time-dependent current: i_{Na}, i_{K1} and $i_{b,Na}$ were not sufficient by themselves to reproduce Weidmann's result). It also showed a slow decline in membrane slope conductance during the pace-maker depolarization. Important though these experiments were (all models

must take them into account and reproduce them) their interpretation has proved quite difficult. The general reasons were reviewed by Noble & Tsien (1972) who gave the equation for the slope conductance as a function of individual ionic currents and their voltage dependence. This equation shows that there is no reason, *a priori*, for assuming that the underlying membrane conductance change responsible for a particular potential change should vary in the same direction as the total slope conductance. Weidmann (1956) clearly realized the importance of this argument in relation to the plateau slope conductance measurements. We shall see later that this general problem has assumed great importance in interpreting the conductance measurements during pace-maker potentials and voltage clamps in the pace-maker range of potentials.

(b) In relation to the pace-maker slope conductance measurements, when Hutter and I (1960) first described our work on inward-going rectification we drew attention to the possibility that a decrease in slope conductance during the pace-maker depolarization could be merely a secondary consequence of the depolarization (via its effect on i_{K1}) rather than a reflexion of the primary cause of the depolarization, which Weidmann's pulse experiments showed must involve a voltage- and time-dependent change. This kind of problem was met by Vassalle's (1966) work in which he measured the change in slope conductance under voltage-clamp conditions. The change he observed was small (about 20 %) compared to Weidmann's measurements, which confirms that a large part of the slope conductance change during the pace-maker potential is voltage-dependent rather than time-dependent, but he still found a decrease in conductance with time under voltage-clamp conditions.

(c) The obvious and then standard interpretation of this conductance measurement was reinforced by the fact that all the measurements of the reversal potential E_{rev} for i_{K2} showed that it varies with $[K]_o$ in the manner expected for a very *specific* K current, i.e. there is a 60 mV change in E_{rev} for a 10-fold change in $[K]_o$ (Noble & Tsien, 1968; Peper & Trautwein, 1969; Cohen, Daut & Noble, 1976). It requires only a fairly small leak in a Goldman-type equation for this slope to fall to, say, 50 or 40 mV.

(d) Also consistent with the view that a pure K current was involved is the fact that the instantaneous $i(V)$ relations for i_{K2} (dissected using the ratio method that Tsien and I described in 1968) display both inward-going rectification and the 'cross-over' phenomenon (in which the net current at different values of $[K]_o$ changes in different directions depending on the voltage range and K concentrations) that are so typical of some K conductance mechanisms (e.g. i_{K1} in the heart, the inward rectifiers in skeletal muscle and in starfish egg cells).

In fact, I think it is fair to say that the weight of evidence for the g_K decay hypothesis in Purkinje fibres was so strong that i_{K2} was regarded as perhaps the most fully analysed current in the heart and served as a model for other systems. One of the surprises to which the title of this review refers is that this interpretation of the pace-maker current in the Purkinje fibre should not only turn out to be deeply incorrect but that a very different interpretation should be able to account for these results both accurately and with no need to suppose that any of the original experimental results is incorrect. Indeed, as I shall show, some important and awkward details of the experimental results then receive very natural explanations.

The second element of surprise implied by my title concerns the analysis of the slow inward current i_{si}. The standard analysis of this current in multicellular preparations is that of Reuter & Scholz (1977). Though this work appeared too late to be incorporated into the MNT model, it nevertheless is in accord with that model in giving i_{si} inactivation kinetics a crucial role in determining the time course of the plateau phase of the action potential. Another important feature of Reuter & Scholz's (1977) analysis is the measurement of the relative contributions of Ca, Na and K ions in carrying the current. They estimated the relative permeability coefficients to be 1:0·01:0·01 (Ca:Na:K) which, given the high concentration of Na and K compared to Ca ions means that as much as 30–40% of the current carried might be carried by ions other than Ca. There are both theoretical and experimental reasons for reassessing these conclusions.

The third element of surprise is one that contributes strongly to the other two: this is that changes in ion concentrations, intracellular and extracellular, have turned out to be much more important, and more rapid, than previous work allowed. A model, like the MNT model, that assumes fixed concentrations is now therefore severely limited, as will become apparent as the story unfolds.

THE SECOND STAGE: THE RECENT REVOLUTION

I call this stage a revolution because it does indeed turn things inside out and upside down, but also because the changes have occurred with great speed. At the end of 1979 it was still reasonable to use models of the MNT kind. By mid-1980 it was already clear that on at least three counts, the model's usefulness was in question. Moreover, the new results with single cells and patch-clamp methods have appeared with great rapidity over the last 3 years. This will be the longest section of my review so, for convenience, I shall divide it into five parts.

1. *The 'pace-maker' current, i_{K2}, turns upside down*

Increasing difficulties with the i_{K2} model

Although the analysis of this current as a pure K current seemed very secure, it is worth recalling that there were some awkward details evident in the results from the very beginning. Eric McAllister and I (McAllister & Noble, 1966) had found that the current disappears in the pace-maker range in Na-free solutions (this is also evident in Deck & Trautwein's (1964) analysis), which is fairly odd behaviour for a highly specific K mechanism. The best explanation we could propose was that the current was perhaps dependent on $[Ca]_i$ which is known to increase greatly in Na-free solutions. Then Dick Tsien and I (Noble & Tsien, 1968) found that the absolute values of the reversal potentials (E_{rev}) were always a few millivolts more negative than expected from the Nernst equation for K ions using a likely value for $[K]_i$. We noted the result and suggested that the problem might lie in trusting intracellular electrode measurements of absolute potential to within 5–10 mV. Peper & Trautwein (1969) also found this result (their Nernst plot requires $[K]_i$ to be 200 mM which is 50 mM greater than has been measured with K-sensitive electrodes (Miura, Hoffman & Rosen, 1977)).

Cohen, Daut and I took up the story again in 1976 (Cohen *et al.* 1976) and fully

confirmed that E_{rev} is always 5–15 mV negative to the calculated E_K. Instead of postulating a systematic error or a high $[K]_i$ we opted for the view that the extracellular cleft K concentration, $[K]_c$, is lower than the bulk extracellular concentration, $[K]_b$, by about 1 mM.

This hypothesis however creates theoretical difficulties, some of which are treated in a review by Attwell, Eisner & Cohen (1979). It is possible to construct a consistent model with a steady-state extracellular concentration difference (see Appendix to Cohen, Noble, Ohba & Ojeda, 1979) but only at the cost of making assumptions about non-uniform distribution of channels and ion pump sites that would be very difficult to test experimentally.

But the necessity for such hypotheses was soon removed and, paradoxically, the work that achieved that result was initially aimed at exploring the contributions of cleft concentration changes in more detail *within* the framework of the i_{K2} hypothesis.

It would require a full length review of its own to explore fully the recent work on extracellular ion accumulation and depletion in the heart. For the present purpose it will suffice to take a short cut with two propositions that I think are now well established.

(1) Given the size of the extracellular space and the magnitudes of the currents flowing in Purkinje fibres it is impossible to avoid the conclusion that, at the i_{K2} reversal potential (about −100 mV), about 0·5–1 mM of extracellular K depletion must occur during prolonged voltage-clamp pulses.

(2) The instantaneous rectifier, i_{Ki}, is extremely sensitive to $[K]_o$ at negative potentials. Thus, at the least, there must be considerable contamination of i_{K2} by changes in i_{K1} due to K depletion.

This was the stage of the story investigated by DiFrancesco, Ohba & Ojeda (1979) who showed that the experimental results on E_{rev} for i_{K2} conform fully to the predictions of these propositions. But that was the opening of the way to the more fundamental move, which was made by DiFrancesco when he returned to work in Italy: if K-dependent changes in i_{K1} can distort i_{K2} to that extent, how do we know anything at all about i_{K2} near its supposed reversal potential? Perhaps it does not reverse at all in that range! Maybe it is not a specific K ion current after all.

DiFrancesco's reinterpretation

And so, i_{K2} was turned upside down. From being a specific K ion channel generating outward current in the pace-maker range it became a non-specific channel generating *inward* current in this range largely carried by Na ions. The hypothesis in its quantitative form (see DiFranceso & Noble, 1982, 1984) demonstrates that what was measured as i_{K2} in fact consists of two components:

$$i_{K2}(t) = \Delta i_{K1}([K]_c) - i_f(t), \tag{1}$$

i.e. a current component due to the inward rectifier, i_{K1}, that varies with time (t) because the cleft K ion concentration varies with time, and a genuine time-dependent gated current, i_f. It so happens that the time constants of K depletion and the gating of i_f are of the same order of magnitude so that the net current records are often, though not always, monotonic and so give the appearance of a single component. Moreover, the voltage range at which Δi_{K1} becomes significant and can mask i_f to

produce a 'reversal potential' varies almost exactly in parallel with E_K since the voltage range of i_{K1} rectification varies in parallel with E_K.

Fig. 3 shows one of DiFrancesco's crucial experiments. First, he obtained i_{K2}-like behaviour (see records on the left). Then he used Ba ions to block i_{K1} (and so massively reduce the problems of K ion depletion). He then obtained the records

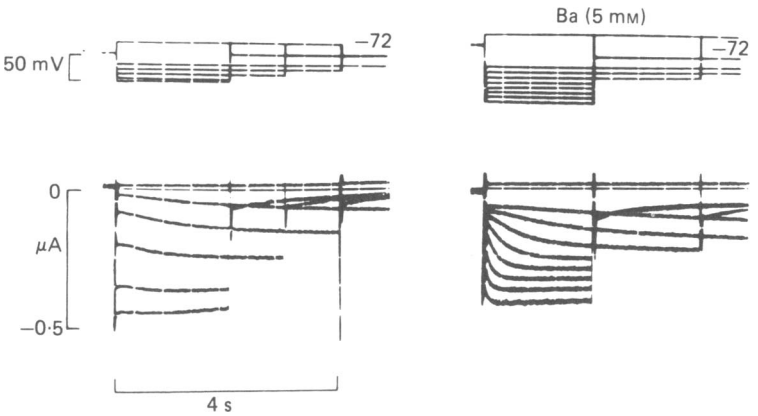

Fig. 3. DiFrancesco's (1981) experiment showing that the 'reversal potential' for the current activated in the pace-maker range of potentials is abolished when i_{K1} is blocked by Ba ions. The records on the left show what happens when the voltage protocol shown in Fig. 2*B* is extended to more negative potentials. The time-dependent current change becomes smaller and eventually reverses. In this case, the reversal potential is situated at −127 mV. This reversal potential is negative to the expected value of E_K but it changes accurately in parallel with E_K when $[K]_o$ is varied (for a review of these results and their explanation see DiFrancesco & Noble, 1982). The records on the right show the currents obtained when 5 mM-Ba is added to the solution. Even though the voltage protocol (see above) is extended to much more negative potentials (down to −165 mV in this case), no reversal potential is observed. DiFrancesco's conclusion was that the reversal depended on K-dependent changes in i_{K1} rather than in the slow time-dependent current (see eqn. (1)).

shown on the right: no reversal potential is then found near −100 mV. He telephoned me from Milano in January 1980 to tell me this result and the same night I was able to use a computer program he and I had developed together to show that his new interpretation of i_{K2} as a non-specific inward current i_f could give a full and accurate theoretical account of the i_{K2} results (we presented this theory at the March 1980 meeting of the Physiological Society (DiFrancesco & Noble, 1980) and later developed it into a full paper (DiFrancesco & Noble, 1982)). Meanwhile, DiFrancesco went on to determine the ionic species (almost equally Na and K) carrying the current i_f (DiFrancesco 1981*a*, *b*) and to analyse the kinetics, which turn out to be significantly different from those obtained when changes in i_{K1} due to K ion depletion are present (Hart, 1983; DiFrancesco, 1984).

DiFrancesco's reinterpretation has been amply confirmed by other experimental work, notably Callewaert, Carmeliet, Van der Heyden & Vereecke's (1982) demonstration that in isolated *single* Purkinje cells, with no restricted intercellular spaces, i_{K2} behaves like i_f even without using a blocker such as Ba ions to eliminate i_{K1}.

The background to the reinterpretation

This reinterpretation of i_{K2} was, without doubt, a big surprise and even more so is the fact that it did not need to cast doubt on *any* of the previous experimental results: on the contrary, it has provided a better explanation for those results since the new theory accommodates the previously awkward results quite naturally. Nevertheless, some of the controversy surrounding the reinterpretation has implied that, at least originally in 1980, it was based on inadequate reasons or on the basis of a single result crucially dependent on a correct interpretation of the action of Ba ions.

I have never felt that, which was why I so readily accepted the full implications of Dario's telephone call from Milano. The reasons are very relevant to the theme of this review so I shall explain them in some detail.

First of all it is important to recall that the current i_f was not discovered first in Purkinje fibres (except under its disguise as i_{K2}). It was first discovered, as a hyperpolarizing activated current, in the s.a. node and since a lot of the work involved was done in the Oxford laboratory it was natural that I and my colleagues should be greatly influenced by the close resemblance to i_{K2} (this resemblance was in fact noted in the 2nd edition of *The Initiation of the Heartbeat* (Noble, 1979, p. 102). Seyama (1976) described inward-going rectification in the mammalian s.a. node and drew attention to the fact that a lot of it was time dependent and was reduced in Cl-free solutions. Brown, Giles & Noble (1977) then described a hyperpolarizing activated current in the frog sinus venosus, though they did not actually prove that this was the correct interpretation. The convincing proof that it was correct came with measurements of conductance changes in the mammalian node (DiFrancesco & Ojeda, 1980) showing that the membrane conductance *increases* with time on hyperpolarization. Noma, Yanagihara & Irisawa (1977) also found the current (which they called i_h) in rabbit s.a. node and Yanagihara & Irisawa (1980) showed that its activation range is very similar to that for the supposed deactivation of i_{K2} in Purkinje fibres, i.e. from -60 to -90 mV. Now the relevant interesting feature of the s.a. node is that, by comparison with the Purkinje fibre, i_{K1} is very weak, i.e. the s.a. node naturally provides precisely the conditions for which Ba was used in the Purkinje fibre. So i_f could be recorded as such (and not under the guise of i_{K2}) even without using blockers. Moreover, like i_{K2} or i_f in Purkinje fibres, i_f in the s.a. node is blocked by low concentrations (1 mM) of Cs ions and is almost eliminated in Na-free solutions (Kimura, 1982). Brown & DiFrancesco (1980) and DiFrancesco & Ojeda (1980), in their papers on the s.a. node, summarized these similarities very effectively even before the reinterpretation of i_{K2}. It is a sign of how much has changed that it is now almost inconceivable that some should have reacted to those papers by finding the analogy far-fetched and implausible.

So, my laboratory had been living already for 3 years with the tensions generated by the uncanny resemblance of the nodal i_f with i_{K2}, and with the increasing theoretical difficulties posed by the i_{K2} model itself. In fact, before Dario and I used the computer program we had developed to test the i_f theory as an explanation for i_{K2}, we had been using it for internal consistency checks on the i_{K2} hypothesis. While he was carrying out the Ba experiment in Milano, I had already satisfied myself that

the i_{K2} hypothesis is internally inconsistent: one cannot have *both* the Cohen *et al.* (1976) explanation for the negative level of the reversal potential *and* the possibility even of measuring a true reversal potential. (This point resembles and is greatly influenced by an argument presented by David Attwell – see Attwell *et al.* 1979; it differs from his argument largely in that Dario and I carried out full computations

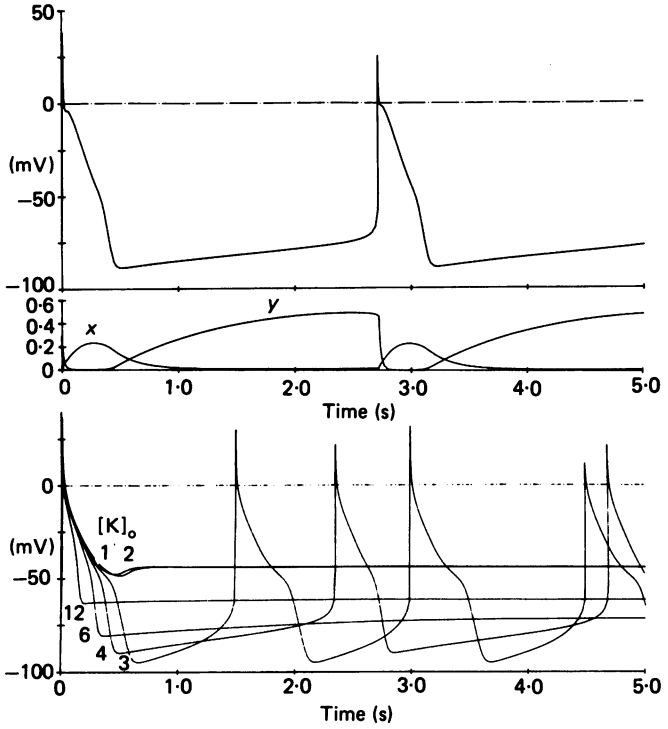

Fig. 4. Reconstruction of Purkinje fibre pace-maker activity using the DiFrancesco–Noble (1984) equations which incorporate DiFrancesco's reinterpretation of the current in the pace-maker range. Top: computed action potentials at 4 mM-external [K] together with the computed variations in the gates controlling i_K (x) and i_f (y). The largest variation during the pace-maker potential is clearly attributable to i_f. This diagram in fact over-emphasizes the role of i_K since at the beginning of the pace-maker depolarization the potential is close to E_K so that i_K is very small even when activated. By contrast, i_f is large in this range of potentials. Near E_K little K flows through the i_f channels so that the major current change is due to Na ion flow through these channels. Bottom: influence of $[K]_o$ on computed pace-maker activity in Purkinje fibres. The figures on each record show the value of $[K]_o$. The model accurately reproduces the K dependence of Purkinje fibre pace-maker activity (Vassalle, 1966) including the acceleration and eventual depolarization in low $[K]_o$ and the suppression of pace-maker activity above 6 mM. This high K sensitivity is attributable to the actions of $[K]_o$ on i_{K1} (cf. Noble, 1965). Thus, while i_f is the main current that *initiates* the pace-maker depolarization, other currents can also play a major role in determining the frequency.

of the influence of a restricted extracellular space with parameters appropriate to a Purkinje fibre, whereas Attwell's argument was presented in general terms of the relevant differential equations.) I had in fact reached almost the same conclusion in some rough calculations discussed with Dick Tsien in 1977 showing that there were

not enough K ions in the extracellular space to carry all the current that flows negative to the supposed reversal potential without postulating massive replacement by diffusion.

DiFrancesco and I never published the theoretical work on the internal inconsistency of the i_{K2} hypothesis because we became too fully occupied with his new hypothesis. But in some ways that is a pity: I think some people would have been less surprised by the revolution and its apparent suddenness if they had fully appreciated the weight of this theoretical evidence and of the sinus node results. Seen in this light, Dario's 1980 experiment came not as an awkward fact destroying a pet theory but rather as a great source of relief: an internally consistent theory could be reconstructed and the s.a. node – Purkinje fibre comparison fell fully into place.

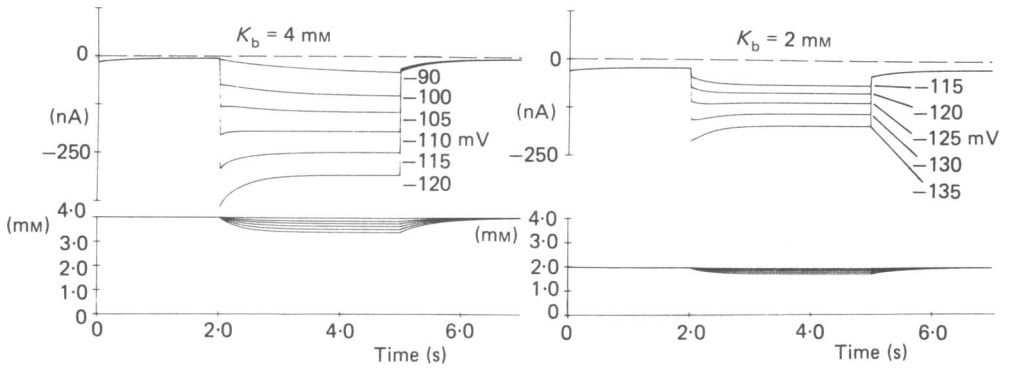

Fig. 5. Reconstruction of the 'reversal potential' for i_{K2} using the DiFrancesco–Noble (1984) model. Results for two values of $[K]_b$ are shown (2 and 4 mM). The model membrane was clamped to the potentials shown from a holding potential of −50 mV. Note reversal of time-dependent current change at −110 when $[K]_b = 4$ mM and −125 when $[K]_b = 2$ mM. The lower traces show the computed mean $[K]_b$ in the extracellular space. Note that only very moderate quantities of K ion depletion are required to produce the result. The change in [K] at the reversal potential is only about 10% of the bulk extracellular concentration.

Role of i_f in Purkinje fibre pace-maker activity

This is not the place to review fully all the experimental work on i_f. For that the reader is referred to DiFrancesco's recent papers (DiFrancesco 1981*a*, *b*, 1982, 1984). I shall deal rather with the implications for the reinterpretation of pace-maker mechanisms. In the case of the Purkinje fibre these are profound. Fig. 4 shows a reconstruction of Purkinje fibre pace-maker activity using the DiFrancesco–Noble (1984) equations. This Figure is based on Figs. 6 and 8 of DiFrancesco & Noble (1984) showing both the large variation of i_f (carried largely by Na ions in this range of potentials) and the large influence of external K concentration on Purkinje fibre pace-maker activity (see Vassalle, 1965; Noble, 1965).

Normal Purkinje fibre pace-maker activity is therefore attributable to the slow onset of a largely Na current during the pace-maker depolarization. Fig. 5 shows computations of ionic current during voltage-clamp protocols of the kind used to analyse i_{K2}. It can be seen that the 'reversal' potential and its K dependence are nicely

reproduced by the new model. For further details on the theoretical comparisons between the i_f and i_{K2} hypotheses the reader is referred to DiFrancesco & Noble (1982). It is however worth noting here that two of the important experimental results that are naturally explained by the new model are Weidmann's (1951) and Vassalle's (1966) conductance measurements. Thus we arrive at the conclusion that the underlying conductance change here *is* in the opposite direction to the over-all measured slope conductance change.

i_f in other parts of the heart

In addition to Purkinje fibres and the s.a. node, i_f has also been found in the mammalian atrium (Earm, Shimoni & Spindler, 1983) and in frog atrium (Bonvallet, personal communication). A current behaving like i_{K2} has also been analysed in embryonic ventricular cells by Clay & Shrier (1981*a*,*b*). DiFrancesco and I (1984) have shown that it is possible that this is also i_f partially masked by changes in i_{K1} due to depletion.

2. *How many 'second inward' currents?*

The second pillar of the recent revolution is the dissection of and finer resolution of the kinetics and amplitude of i_{si}. There are some rough analogies with the reinterpretation of i_{K2}. First, what was initially described as a single current mechanism turns out to be a composite of at least two, and even three, processes. Secondly, one of these processes very probably may involve substantial variations in ion concentrations rather than in voltage-gated ion channels. And thirdly, the kinetics of the largest current mechanism involved in recent work are very considerably different from those of the largest component usually detected in previous work. But there are also some important differences: the main current component involved is still thought to be carried by Ca moving inwards across the cell membrane. The extreme view that most or all of i_{si} is due to processes like the Na–Ca exchanger or Ca-activated channels is clearly incorrect: though I shall show that such processes may contribute significantly, the major part of i_{si} continues in one form or another to consist of Ca inflow through membrane channels. I have already shown (see Fig. 1) the great diversity of forms that i_{si} has taken in experimental work even before the introduction of single cell work. The inactivation time course has been variously estimated as having a time constant as brief as 20 ms (Rougier *et al.* 1969) and as long as 500 ms (Beeler & Reuter, 1970). Sometimes inactivation appears to be much slower than activation: sometimes (as in Fig. 1*C*) the current appears to take as long to activate as to inactivate. Could all these diverse wave forms really be manifestations of the same mechanism?

My approach here will be, first, to review the recent evidence on i_{si} obtained in work on the multicellular s.a. node preparation and on single cells from various sources. I will then reinterpret previous work in the light of these more recent experiments. The approach will be rather more piecemeal than for the i_f/i_{K2} story since, as yet, there is no generally accepted theoretical structure within which *all* the new results can be accommodated. The DiFrancesco–Noble (1984) equations, however, do provide a partial reinterpretation and I shall use these where I think they help to clarify the situation. I should though emphasize the more speculative nature of some of what I am going to say in this section of the review.

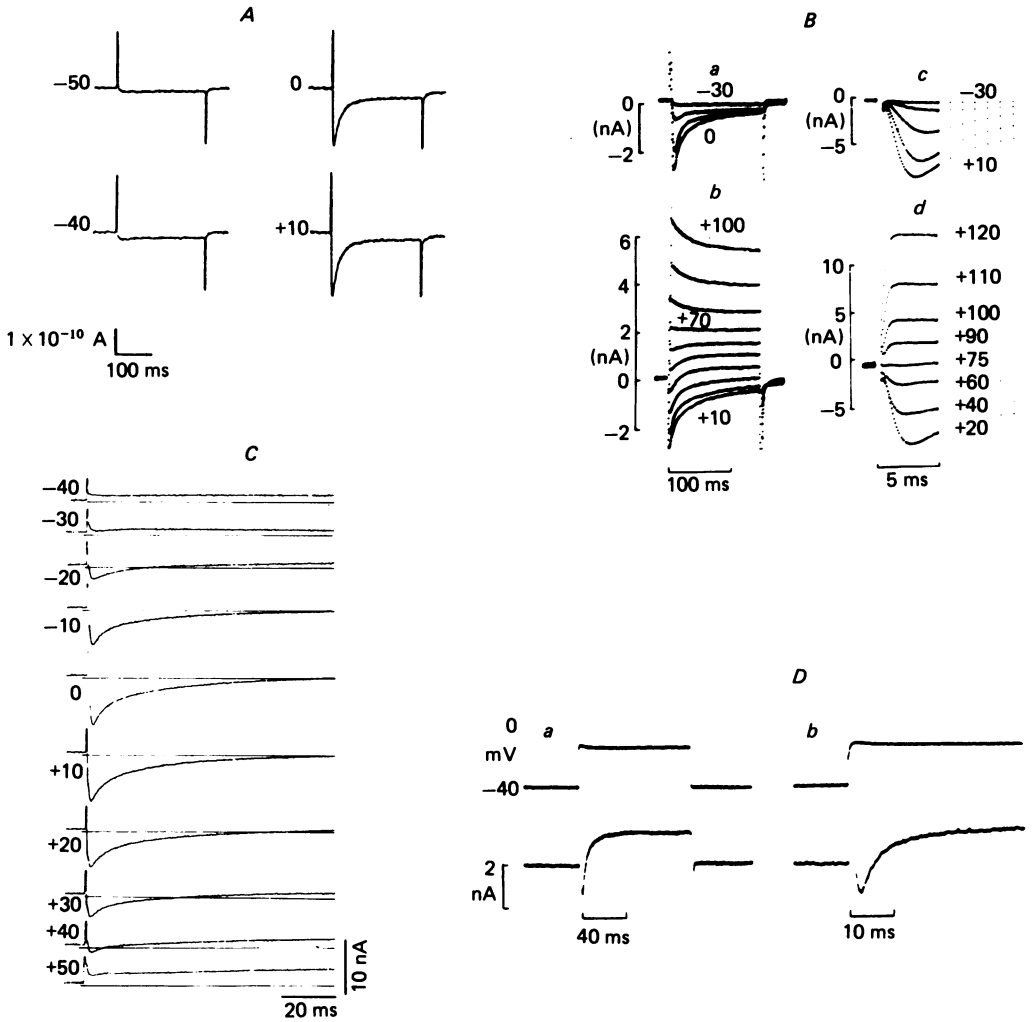

Fig. 6. 'Fast' second inward currents recorded in isolated single cardiac cells. *A*, atrial cells from the bull-frog (Hume & Giles, 1983). The membrane was clamped from −80 mV to the potentials shown. The first inward current is a *maintained* inward current at −50 and −40 mV. This current is resistant to the fast Ca channel blocker Cd and will be discussed, together with a similar current found in guinea-pig ventricular cells, in a later section of this review. Stronger depolarizations (examples here at 0 and +10) show a fast current that reaches a peak in less than 5 ms and a time constant of inactivation around 20 ms. The maintained current is also still present. *B*, perfused guinea-pig ventricular cells (Lee & Tsien, 1982). The records in *a* show currents recorded up to 0 mV and those in *b* show the currents at positive potentials. Note that a reversal potential occurs at +70 mV beyond which outward (probably K) current flows through the channel. The expanded records in *c* and *d* show that the current reaches a peak at about 2–3 ms. The time constant of inactivation is about 20 ms. *C*, bovine ventricular cells (Isenberg & Klockner, 1982). The potential was stepped from −50 mV to the potentials shown. Peak activation time is around 2 ms and inactivation time constant near 20 ms. *D*, rat ventricular cells (Mitchell *et al.* 1983). The potential was stepped to 0 from −40 mV. The inward current peaks at 2–3 ms and inactivates with a time constant near 10 ms. Thus, in a wide range of cell types, the kinetics of the fast Ca current (called $i_{Ca,f}$ in this review) are very similar. This suggests that the enormous diversity of time courses found in previous multicellular work is *not* attributable to large natural variations in the speed of the same mechanism.

The main component of i_{si} is very fast

It is convenient to start with the single-cell work. In any case, this is likely to be more reliable since voltage-clamp control is more rapid and probably more uniform in single cells than it is in multicellular preparations, even if very small as in the case of the s.a. node. The striking and uncontrovertible feature of the single-cell results is that the main part of what is *now* described as i_{si} is far from being a *slow* inward current. It is *much* faster and considerably larger than previous work suggested (see, e.g. discussion in Mitchell, Powell, Terrar & Twist, 1983, for details) and these facts have considerable importance for the reinterpretation of the role of the second inward current in normal electrical activity. Fig. 6 shows a selection of results taken from various laboratories using the single-cell technique to study i_{si}. It is clear that peak activation of the current occurs within 2–5 ms rather than 20–100 ms and that the inactivation time constant is in the range 10–20 ms rather than hundreds of milliseconds or even seconds. Thus these figures give kinetics at least an order of magnitude faster than most of the multicellular work suggested. This immediately raises several important questions.

(1) Were the much slower currents recorded in multicellular preparations distorted versions of the current recorded in single cells, or is it rather that the single-cell work has fully revealed a fast component that was greatly masked in the multicellular work, and that there are also slower components which formed a proportionately greater part of the recorded current in multicellular preparations?

(2) If there are several components, are they all gated channels or could some of the current be attributed to carrier processes and their variation with ion concentrations?

(3) What are the implications for the role of i_{si}, or its components, in the generation of the plateau, in repolarization and in pace-maker activity?

Before we proceed it is important to establish some consistent terminology. I shall now use the symbol i_{si} to refer to the *total* slow inward current. This convention is consistent with the fact that most previous work uses i_{si}, though usually without recognizing that it may not be a single component (though it is worth noting that the MNT model used two components, one of which was a maintained, non-inactivated, current). Moreover, even in the single-cell work we still need a term for the over-all current since it is not always possible to dissect the components. For the *fast* component of current identified in the single-cell work I shall use the symbol $i_{ca,f}$. This terminology has not been used before but as my review progresses it will become obvious why I need a separate term. We shall also require new terminology for any slower components. I shall introduce this later.

First, then, we deal with the question whether slower currents recorded in multicellular work could be artifactually slow recordings of the fast current, $i_{Ca,f}$. A possible mechanism for this would be slowing of the current record due to the presence of a series resistance between the cell membranes and the current collecting electrode (Isenberg & Klöckner, 1982; Noble & Powell, 1983). This approach looks plausible initially since Isenberg & Klöckner (1982) tried the effect of inserting a series resistance of up to 10 MΩ on their single-cell current recordings. This does indeed increase the time to peak but not to much more than 10 ms. Powell and I (Noble & Powell, 1983)

used the DiFrancesco–Noble (1984) equations, which use kinetics for i_{si} based on the fast single-cell kinetics. We found that increasing the series resistance to 10 MΩ could delay the peak to about 6 ms but the amplitude would then become very small (only about 20 % of the true value). A peak delayed by much more than this would become negligible. Now it is indeed the case that the peak amplitude of i_{si} is much larger in single cells than it is in multicellular preparations (when due allowance has been made for the different membrane areas) (see Isenberg & Klöckner, 1982; Mitchell *et al.* 1983). It is likely therefore that i_{si} (and in particular $i_{Ca,f}$) has been seriously underestimated in the older work.

Another factor that would lead to an underestimate of the amplitude of i_{si} is masking by the transient outward current. This would be particularly serious in Purkinje fibres where Siegelbaum & Tsien (1980) were able to show that much faster and larger inward currents could be recorded when i_{to} had been removed by injecting the preparation with EGTA to buffer the Ca ion concentration and so eliminate the $[Ca]_i$ transient. This important experiment (Fig. 1 *B*) shows that a large and fast part of i_{si} is indeed masked by a Ca-activated outward transient. It may also be the case that the Ca buffering may have eliminated slower components of i_{si}.

It is probable, I think, that both series resistance 'blunting' and outward current 'masking' are at work. But it is also, I think, clear that this cannot be the full explanation of relatively slow and small inward currents in multicellular preparations. There are two reasons for this view. First, the EGTA injection experiments of Siegelbaum & Tsien show that, even if inward currents quite as fast as in single cells cannot be obtained in a multicellular preparation, nevertheless, once i_{to} is removed, the inward current is fairly fast, and reaches a peak in about 5 ms. Moreover, the inactivation time constant is also fairly short. In other words, with the $[Ca]_i$ transient removed by EGTA, the inward current record *approaches* that obtained in the single-cell work. There is no reason to suppose that EGTA injection should alter the extracellular series resistance. Series resistance problems cannot therefore be responsible for much slower currents with times to peak as long as 50 ms. The more reasonable explanation is that such currents also exist, are small compared to $i_{Ca,f}$ and are obtained most readily when most of $i_{Ca,f}$ is masked by the transient outward current. The second reason for this view is that the computations that Trevor Powell and I did show that substantial slow inward currents peaking at about 50 ms could not be generated from $i_{Ca,f}$ by series resistance artifacts since the series resistance required would be implausibly large and the current amplitude much too small.

A very slow transient component of i_{si}

But if there really do exist additional components of inward current then why not look for them directly? This has been done both in single cells and in the multicellular s.a. node preparation. The work on the s.a. node was part of a project to assess quantitatively the contribution of different ionic currents to the generation of the normal rhythm of the heart. i_{si} is one of the currents that has been proposed as the main 'pace-maker' current so it seemed important to analyse its behaviour during the pace-maker depolarization. It was during such an analysis that the discovery was made that if one applies a voltage clamp at the potential reached towards the end of the pace-maker depolarization (about −45 mV) the inward current recorded

sometimes shows the time course illustrated in Fig. 7. This very clearly resembles the Purkinje current recorded at a similar voltage in Fig. 1*C*, i.e. activation and inactivation take almost equal periods of time and the peak inward current is not reached until 50–100 ms after the onset of the clamp. Here there is no question of the delay being produced by the time taken for the voltage step to occur via current flow through a series resistance since there is no voltage step. The preparation had

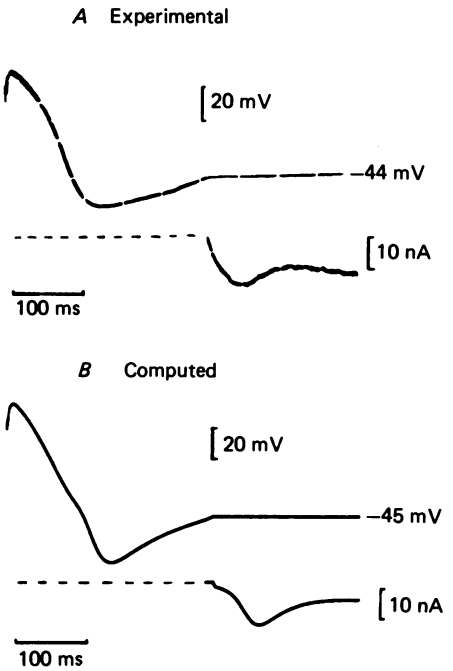

Fig. 7. The current, $i_{si,s}$, in rabbit s.a. node. The top records show one way of obtaining this current with little contamination from $i_{Ca,f}$. The membrane potential is allowed to follow the natural time course of the pace-maker depolarization until a potential near −40 to −50 mV is reached. The voltage is then clamped at this point. There is no voltage step and no transients attributable to a step. In some experiments, as here, a very slow inward current develops. The time course resembles that of Fig. 1*C* since activation and turn-off take almost equal periods of time. The bottom records show a reconstruction of this type of current record using the s.a. node version (Noble & Noble, 1984) of the DiFrancesco–Noble (1984) equations. The current here is computed using a Mullins-type model for the Na–Ca exchanger assuming a stoicheiometry of 3:1. (From Brown *et al.* 1983, 1984*a*.)

already naturally achieved the voltage required. A further argument for the view that such current records are not artifactual is that it is sometimes possible in voltage-clamp experiments in the s.a. node to distinguish two separate peaks of inward current. An example of this is shown in Fig. 8*A*.

It is of importance to note that the separation is only rarely as complete as this and that there is only a fairly narrow voltage range over which two peaks can clearly be seen. This is typically in the range −50 to −30 mV. At more negative potentials, no inward currents are recorded (i_{Na} has been blocked with tetrodotoxin (TTX)). At more positive potentials, the slower component speeds up and fuses with the faster

one to give the appearance of a single component. In some experiments it does this at all potentials.

This behaviour is not specific to the mammalian s.a. node. In the presence of 2 mM-4-AP to block i_{to}, two peaks of inward current can also be recorded in this voltage range in Purkinje fibres (G. Hart & D. Noble, unpublished). But perhaps the

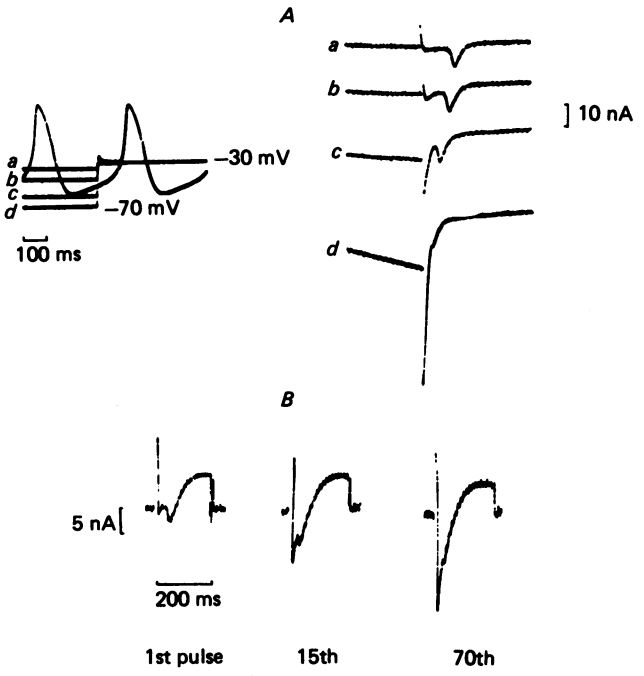

Fig. 8. This shows two other protocols that sometimes reveal $i_{si,2}$ in the rabbit s.a. node. *A*, left: voltage steps superimposed on a record of natural pace-maker activity. Right: current records. Note that as the holding potential is made more negative, progressively more $i_{Ca,f}$ is activated. Following this a very slow current similar in time course to that shown in Fig. 7 occurs. The time delay between $i_{Ca,f}$ and $i_{si,2}$ decreases as more $i_{Ca,f}$ is activated. Our interpretation of this result is that the speed of onset of $i_{si,2}$ depends on the speed of Ca entry and subsequent intracellular release This record is unusual in showing such a complete separation between the two components. More usually, $i_{si,2}$ appears as a slow tail following on $i_{Ca,f}$, as in the record in *d*. This is also how we think it usually occurs during natural pace-maker activity (see computer reconstruction in Fig. 12). (From Brown *et al.* 1984*a*.) *B*, two components of i_{si} recorded during a train of depolarizations (from −65 to −30 mV) following a long period of rest. The total current shows the 'staircase' phenomenon (cf. Noble & Shimoni, 1981). As in *A*, when $i_{Ca,f}$ becomes larger, the delay between this and $i_{si,2}$ decreases and the two components fuse together. (Reprinted with permission from Brown *et al.* 1982, 1984*a*.)

most convincing evidence comes from work on isolated single guinea-pig ventricular cells (Lee, Lee, Noble & Spindler, 1983), where it is possible not only to record the two peaks but also to show that when the first peak, i.e. $i_{Ca,f}$ is rapidly blocked by 0·2 mM-Cd ions (Lee & Tsien, 1982), the second peak remains for a few minutes. The second peak is strongly correlated with the contractile activity and, when, as is sometimes the case, this is oscillatory, the very slow inward current is also

oscillatory. In its time course and in the fact that it is sometimes oscillatory, this current strongly resembles the transient inward current, i_{TI}, first described in Purkinje fibres by Lederer & Tsien (1976). i_{TI} has also been shown to correlate strongly with the contractile properties of the preparation (see review by Tsien, Kass & Weingart, 1979). Lederer & Tsien used preparations in which the Na–K exchange pump was blocked by cardiotonic steroids to create the conditions (raised intracellular Na and, therefore, raised intracellular Ca) required to generate the after-contractions with which the current is correlated. Eisner & Lederer (1979) subsequently showed that Na-pump blockade produced by using K-free solutions was equally effective. But, of course, during a normal twitch the intracellular Ca may rise to values even greater than that occurring during after-contractions produced by Na-pump blockade. There is therefore no surprise in finding that a similar current may be activated during normal electrical activity.

What produces it? So far there are two theories, both of which were discussed by Kass, Lederer, Tsien & Weingart (1978). The first is that i_{TI} is the inward current generated by an electrogenic Na–Ca exchange process extruding Ca ions (which explains why high levels of intracellular Ca are required) in exchange for an electrically greater quantity of Na ions (which would explain why i_{TI} disappears in Na-free solutions). The second theory is that the current flows through non-specific (as between Na and K ions) channels activated by intracellular Ca ions. Such a channel has been found in patch-clamp studies (Colquhoun *et al.* 1981). I shall refer to the Na–Ca exchange current as i_{NaCa} and the non-specific channel current as i_{NaK}. When wishing to remain neutral as between these hypotheses (and any other hypotheses) I shall use the symbol $i_{si,2}$ for the current recorded experimentally. While using this neutral terminology I do nevertheless think that the resemblance of the current recorded in the s.a. node and in single guinea-pig ventricular cells to the original i_{TI} is so strong that a provisional identification of the currents as the same mechanism is justified.

How can we distinguish between the proposed mechanisms? There are several possible lines of argument here.

(1) If $i_{si,2}$ (or i_{TI}) is generated by the non-specific current, i_{NaK}, it should display a reversal potential near 0 mV, where the Na and K gradients are nearly equal and opposite and where the current i_{NaK} has been found to reverse in patch-clamp studies. The early work on i_{TI} in Na-pump-blocked preparations does describe such a reversal potential (Kass, Tsien & Weingart, 1978). By contrast, i_{NaCa} should show more complex behaviour depending on the way in which voltage and the $[Ca]_i$ transient combine to control current flow via the exchanger. This is complicated, but it is possible to show, using the DiFrancesco–Noble (1984) equations, that, at the least, i_{NaCa} should become smaller as the voltage is made more positive (see Fig. 16 and D. DiFrancesco, G. Hart & D. Noble, in preparation). A genuine reversal though of the time-dependent current should not occur since the direction of *change* of current should depend on the direction of change of $[Ca]_i$ at all voltages (this point needs to be clearly distinguished from the fact that i_{NaCa} itself must have an absolute reversal potential, which might not be too different from that for i_{NaK} at resting Ca levels (see Mullins, 1981). The problem is that we are not only dealing with elevated Ca levels, we are dealing with 'reversal' of the current *change* with time. Thus, an outward exchange current might still generate an 'inward' *change* of current level

if $[Ca]_i$ reduces, as it should, the level of such an outward i_{NaCa}, whether or not it succeeds in making it become absolutely inward. The absolute instantaneous reversal potential for i_{NaCa} is therefore irrelevant to the present discussion.

At first sight, the existence of a reversal potential for i_{TI}, therefore favours i_{NaK} as the mechanism. But here I will be frank and reveal my prejudice: I suspect that the 'reversal potential' for i_{TI}, is not always a genuine channel reversal potential. There are several reasons for my suspicion: (a) Karagueuzian & Katzung (1982) and Per Arlock & Katzung (1982) failed to find a reversal potential for i_{TI} in work on mammalian ventricular muscle; (b) even in Purkinje fibres, the reversal is sometimes fairly odd, as shown for example in the current–voltage diagrams for i_{TI} published recently by Hennings & Vereecke (1983): the current falls towards zero between −40 and −30 mV and then *stays at zero* for about 30 mV before showing a very small reversal. This looks much more like a voltage *range* over which i_{TI} is negligible or non-oscillatory (cf. Fig. 16) rather than a specific channel current reversal potential; (c) in work on i_{TI} in the s.a. node we have sometimes found that as the range of the presumed reversal potential is approached the oscillation shows a phase shift (Brown, Noble, Noble & Taupignon, 1984) which would imply that the phase of the $[Ca]_i$ oscillation had changed. A 'reversal' in such an experiment might then be no more than a combination of (i) a voltage range over which the oscillatory *change* in current is very small, and (ii) a phase shift that enables an outward phase of one oscillation at positive potentials to appear coincident in time with the inward phase of an oscillation at negative potentials.

At the least, therefore, I think the argument based on the existence of a reversal potential for i_{TI} is equivocal.

I should though make it clear that I am not proposing that i_{TI} *never* shows a specific reversal potential. The original experiments of Kass *et al.* (1978) clearly show such a reversal and this result, even if it is not that always obtained, needs to be explained. I will return to this problem below.

2. If i_{TI} is indeed activated (as $i_{si,2}$) during normal electrical depolarizations (as the s.a. node work and the results on isolated guinea-pig ventricular cells suggests) then it is relevant to ask which mechanism would be most compatible with the known behaviour of the action potential. This question is easy to answer. i_{NaCa} is perfectly compatible with the generation of normal ventricular action potentials in which the rate of repolarization is *smallest* at a time (e.g. about 50–100 ms) when the $[Ca]_i$ transient is maximal since the $[Ca]_i$ transient will either activate an inward i_{NaCa} or reduce an outward i_{NaCa}, in both cases assisting the depolarization and therefore the plateau of the action potential. By contrast i_{NaK} activation would distort the repolarization phase in exactly the same way as the end-plate current distorts the skeletal muscle repolarization phase, by always driving the potential towards the current reversal potential (Fatt & Katz, 1951).

There is evidence that at high $[Ca]_o$ and high frequencies this does indeed happen in frog ventricle (Niedergerke & Page, 1982) but it can hardly happen in ventricular action potentials showing the *lowest* rate of repolarization at the time of the *peak* $[Ca]_i$ transient. This argument has been given a quantitative form recently in the DiFrancesco–Noble (1984) equations in which i_{NaCa} supports a normal action potential while i_{NaK} distorts it.

This is a convenient point at which to return to the i_{TI} reversal potential measured

by Kass *et al.* (1978). Their current–voltage diagram differs from that obtained by Per Arlock & Katzung (1982), by Hennings & Vereecke (1983) and in our own work since they found a *specific* reversal potential rather than an extensive *range* of zero current potentials or phase shifts. Moreover, in their experiment they excluded the phase shift argument I have presented above since they recorded the contraction, which, like the current, did not show a significant phase shift. Clearly then, two or three significantly different types of result can be obtained. I think the simplest explanation for this is that, in addition to activating the Na–Ca exchanger, the Ca transient also sometimes activates the non-specific current channels. i_{TI} might then be a composite current, which would be entirely dominated by the non-specific current over the voltage range at which the exchanger current would be expected to fall towards zero. This happens to be in the range of the reversal potential for the non-specific current channel. In conclusion then, the voltage dependence of i_{TI} shows three forms: reversal at a specific membrane potential, a voltage range of zero current change, and a voltage range of combined low amplitude and phase shift of the oscillation. I doubt whether this diversity can succumb to a single component explanation and I therefore propose that the best explanation is that i_{NaCa} and i_{NaK} are activated by internal Ca to different extents in different preparations and experimental conditions. When i_{NaK} is very small or absent, there will be no specific reversal potential.

(3) The third line of argument is more purely theoretical. It is easy to show that if i_{NaCa} is the main mechanism by which Ca leaves the cell then for exchange ratios of 4:1 or 3:1 (the currently favoured Na:Ca exchange ratios) the time integral of i_{NaCa} must be either of the same order of magnitude as that for i_{Ca} or about half this magnitude. This argument is most readily appreciated for a 4:1 exchange since in that case the inward current carried by the excess Na ions must then equal the charge on the Ca ions transported. Now I think it is significant that the slow current $i_{si,2}$ recorded in the s.a. node and in guinea-pig ventricular cells is of the right order of magnitude. Fig. 7*B* shows that the DiFrancesco–Noble (1984) equations, as modified for the s.a. node (Noble & Noble, 1984) can readily account quantitatively for $i_{si,2}$. This model does assume that the Na–Ca exchanger is the main route by which Ca ions leave the cell and that the exchange ratio is 3:1. The Purkinje version of the equations also succeed in reproducing the very slow inward current recorded (see, e.g. Fig. 1*B*) in Purkinje fibres (DiFrancesco & Noble, 1984).

My conclusion, therefore, is that, at present the arguments favour the view that, in *normal* electrical activity, $i_{si,2}$ (or i_{TI}) is largely i_{NaCa} rather than largely i_{NaK}, but I would readily admit that this view could greatly benefit from more direct ways of demonstrating the existence of i_{NaCa} in a way analogous to the elegant experiments of Gadsby (1980), Eisner & Lederer (1980) and Daut & Rüdel (1982) showing the existence of the Na–K exchange pump current, i_p, in the heart. Some recent evidence on i_{NaCa} has been produced by Horackova & Vassort (1979), Mentrard & Vassort (1983) and by Jacob, Murphy & Lieberman (1983) using experimental conditions in which blockers or substituted solutions are used to eliminate all other possible currents.

There is a final point about $i_{si,2}$ which needs to be made to complete the picture as I now see it. I have so far described it as a very slow transient because that is how it appears when it is dissectable from $i_{Ca,f}$ in the voltage range between -60 and

−30 mV. It is important, however, to realize that it is very easy to miss seeing the current for two reasons. First, to obtain a large slow current in this voltage range it is usually, though not always, necessary to use a sufficiently negative holding potential. We used −80 mV in our single-cell work, which is much more negative than is usually used in studies of i_{si}. This requirement may depend on repriming of the internal Ca-release mechanism, which is known to be voltage dependent (Gibbons & Fozzard, 1975). The second reason is that, in both the s.a. node work and the single ventricular cells, as the membrane voltage is made more positive, and $i_{Ca,f}$ is more strongly activated, $i_{si,2}$ becomes considerably faster and then fuses more completely in its time course with $i_{Ca,f}$ to give the appearance of a single component. This behaviour is consistent with a mechanism attributable to i_{NaCa} and is seen also in the behaviour of $i_{si,2}$ in the DiFrancesco–Noble (1984) equations. The mechanism is that as $i_{Ca,f}$ is increased, the Ca transient that is supposed to activate i_{NaCa} rises more rapidly. Depolarization also reduces the magnitude of i_{NaCa} by approaching its reversal potential. Thus, the protocols for separating $i_{si,2}$ and $i_{Ca,f}$ have to be chosen with care and they may well vary in different cells and in different experimental conditions. Amongst the latter, one that we have found to be very important is the condition of the isolated cells. If this is not good enough for them to display action potentials with long plateaus, the slower components of current are difficult to detect. This is not suprising in view of the likely functional importance of the slow components (see below).

A Cd-resistant maintained component of i_{si}

This discussion of additional slow inward currents may already have exhausted the reader, but it certainly does not exhaust the mechanisms that have been found. I referred earlier to the fact that $i_{si,2}$ (the i_{TI}-like current) has been found in single guinea-pig ventricular cells (Lee *et al.* 1983) and that it can be observed for a period of time even when $i_{Ca,f}$ has been blocked by Cd ions. Eventually, after a minute or two in Cd solution, the contractile behaviour changes and $i_{si,2}$ also disappears. In these conditions though, there *still* exists an inward current which is totally abolished when Ca ions are removed (Lee, Lee, Noble & Spindler, 1984*a*). This current, whose peak amplitude is only about 20 % of $i_{Ca,f}$, shows very slow inactivation and at some potentials (around −50 to −30 mV) it hardly inactivates at all. We have called this component $i_{Ca,s}$ since we think it is a very slowly inactivated Ca current. Although much smaller than $i_{Ca,f}$ it is just large enough to support a prolonged action potential with a very slow rate of rise and no overshoot (see Fig. 9).

An inward current very similar to $i_{Ca,s}$ in the guinea-pig ventricle cell has also been recorded recently in isolated single frog atrial cells by Hume & Giles (1983). Like $i_{Ca,s}$ it starts to activate in the region of −60 mV, is resistant to Cd ion block, shows very little inactivation at some potentials and carries a peak current equal to about 20 % of the total i_{si}. There is however an important difference, which is that, whereas in guinea-pig cells $i_{Ca,s}$ promptly disappears in Ca-free solution, the persistent inward current in atrial cells does not (see Hume & Giles, 1983, Fig. 12). It requires many minutes of Ca-free superfusion to be eliminated. Hume & Giles note the resemblance of their persistent inward current to persistent Ca currents found in *Helix* neurones (Eckert & Lux, 1975; Akaike, Lee & Brown, 1978) and vertebrate motoneurones (Schwindt & Crill, 1980) and do not exclude the possibility that their current is also

a Ca current since brief superfusion with Ca-free solution may not remove all the Ca that may be involved.

The existence of $i_{Ca,s}$ should not come as too great a surprise. Ca currents of different inactivation speeds also exist in other cells (see recent review by Tsien, 1983) and the work on multicellular preparations (e.g. Beeler & Reuter, 1970) shows that some component of Ca current must exist that requires several hundred milliseconds for inactivation. Indeed it seems likely that a very large part of the 'slow inward current' recorded in multicellular preparations must consist of currents like $i_{si,2}$ and $i_{Ca,s}$ rather than $i_{Ca,f}$ as I have defined it in this review.

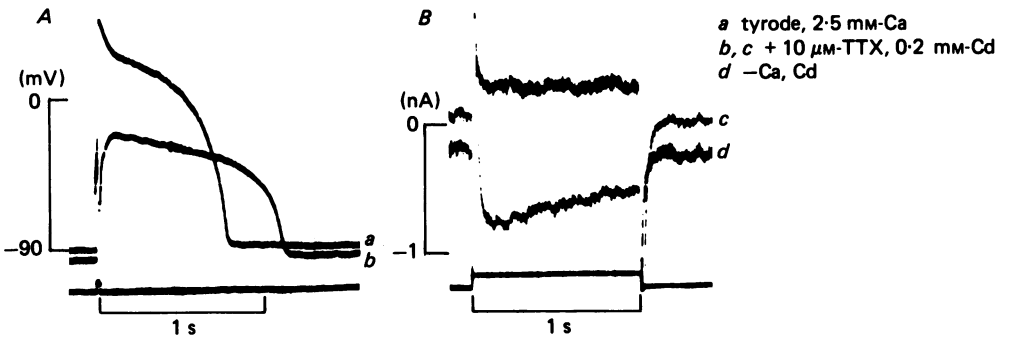

Fig. 9. The very slow Cd-resistant current in single guinea-pig cells. *A*, this shows action potentials recorded before and after application of 0·2 mM-Cd. Note that, even when $i_{Ca,f}$ is blocked by Cd, a long plateau at a lower membrane potential can still be obtained. *B*, this shows ionic current in Cd solution before and after removal of Ca ions. The action potential plateau is also then completely abolished (not shown here). The persistent inward current (which we have called $i_{Ca,s}$) differs markedly from $i_{Ca,f}$ in its voltage range (threshold for $i_{Ca,s}$ is 20–30 mV more negative than for $i_{Ca,f}$), time course (very slow), resistance to Cd, absence of effect of isoprenaline, and absence of Ca-induced inactivation (at some voltages the current is hardly inactivated at all). Together with $i_{si,2}$ (which is also found in single guinea-pig cells (Lee *et al.* 1983)), $i_{Ca,s}$ is thought to generate the very long plateau. (From Lee, Lee, Noble & Spindler, 1984*a*.)

To conclude this rather difficult, and doubtless more controversial part of my review, I propose that i_{si} is composed of three components, which, using completely neutral terminology, might be referred to as $i_{si,1}$, $i_{si,2}$ and $i_{si,3}$. Provisionally, at least, the evidence that the first and third components are Ca channels leads me to replace this neutral terminology with $i_{Ca,f}$ and $i_{Ca,s}$. We then arrive at the dissection:

$$i_{si} = i_{Ca,f} + i_{si,2} + i_{Ca,s}, \tag{2}$$

and it will also be clear that my prejudice on the composition of the second component would lead to the hypothesis of eqn. (3):

$$i_{si} = i_{Ca,f} + \Delta i_{NaCa} + i_{Ca,s}. \tag{3}$$

The idea that there may be such a mixture of currents would also readily explain the diversity of opinion on the extent to which Na and Ca ions are responsible. $i_{Ca,s}$

seems to be purely Ca (it completely disappears in Ca-free solution) while currents like i_{NaCa} (or i_{NaK} if this should turn out to be the correct explanation for $i_{si,2}$) should disappear in Na-free solutions.

Correlations with multicellular work

At this point I am tempted to speculate on the correlation between this dissection of i_{si} based on the single-cell and s.a. node work and the currents recorded in previous work. In some cases, I think we can be fairly certain of the correlations. Thus, it is likely that the first i_{si} recorded by Reuter (1967) in Purkinje fibres (see Fig. 1*A*) contained virtually no $i_{Ca,f}$ since it reached a peak at a time when most of the fast current would be already inactivated. From which one would conclude that it was largely components like $i_{Ca,s}$ and $i_{si,2}$. Similarly, i_{TI}-like currents of the kind shown in Fig. 1*C* are likely to be largely $i_{si,2}$, with some persistent contribution from $i_{Ca,s}$. The much faster currents (Fig. 1*B*) recorded, e.g. by Siegelbaum & Tsien (1980) and by Marban & Tsien (1982) must have contained much more $i_{Ca,f}$ and, with intracellular Ca buffered, we may guess that $i_{si,2}$ was absent. Inward current with fast ($\tau = 20$ ms) inactivation time constants was also recorded in multicellular work on frog atrial trabeculae (Rougier *et al.* 1969). And so one could go on through the past literature. It would, however, be disingenuous of me to suggest that drawing out these correlations is all that easy. There are also some awkward problems. For example, the voltage range of the activation curve for $i_{Ca,s}$ in single cells is appreciably more negative (about -60 mV) than the accepted 'threshold' (about -40 mV) for activating i_{si} in multicellular preparations (Reuter, 1967; Beeler & Reuter, 1970; Reuter & Scholz, 1977). All I think we can say for certain is that there would have been an even greater problem if the single cell work had *not* shown the existence of slower components of inward current.

There is another reason for being cautious about the interpretation of the work on multicellular preparations in the light of that on single cells, particularly when comparing the presumed charge carriers for various currents. This is that it is far from clear what the relative permeabilities for various ions should be. Reuter & Scholz (1977) attempted to measure these by using reversal potentials interpreted using a form of Goldman equation. The equation they used is in fact incorrect, as is the 'corrected' version they published later (see Attwell & Jack (1978) for a correct derivation of equations for channels conducting divalent and monovalent ions simultaneously, and for an important discussion on the ambiguity of using constant-field type models for defining permeabilities in such cases). The conclusion that the i_{si} channels carry Na and K ions as well as Ca ions needs reassessment, both for this theoretical reason and because it is now important to assess the ion carriers *separately* for each of the components of i_{si}. In the case of $i_{Ca,f}$, Lee & Tsien (1982) have shown that outward current (presumably carried by K ions) can be recorded positive to the reversal potential. By itself, given the problems that may arise from current 'masking', this would not be sufficient. They were therefore careful to show that this outward current flow is blocked by Ca current blockers and that there is no change in current level at the reversal potential during such a block. Hume & Giles (1983) made the same observation on current recorded beyond the reversal potential in the case of the fast channel in frog atrial cells. They also investigated the contribution

of Na ions and concluded that Na^+ does not contribute to the fast or persistent inward currents. Mitchell *et al.* (1983) have also concluded that Na ions do not contribute to the fast Ca current in single rat ventricular cells. Yet, the work on i_{si} in multicellular preparations repeatedly suggests that Na ions are involved. If neither $i_{Ca,f}$ nor $i_{Ca,s}$ conducts Na ions, what does? Mitchell, Powell, Terrar & Twist (1984) describe a 'late' plateau current in rat ventricular cells that is Na sensitive. Whether this is identical with what I have called $i_{si,2}$ in this review remains to be seen. Certainly, if $i_{si,2}$ were to be carried either by the Na–Ca exchanger or by the Ca-activated non-specific channels, it would be Na sensitive. Another problem in this connexion is that Ca ion channels may only conduct Na ions in Ca-free solutions.

Before I leave the question of the components of i_{si}, there are some semantic problems that arise. If the 'dissection' I have proposed here is correct (and it is by no means the only 'dissection' that has been proposed – see Isenberg (1983) for an interesting alternative based on distinguishing between sarcolemmmal i_{si} and current that may be carried by internal membrane systems), then it is clear that until very recently the single-cell work has concentrated on what I have called $i_{Ca,f}$. Because it was by no means evident initially whether i_{si} was a single or multicomponent current, the symbol i_{si} tends to be used *both* for the fast current recorded in single cells *and* for the slower currents seen until recently only in multicellular preparations. The impression given is that the same current is being referred to. This is doubly unfortunate from a semantic point of view. First, because it is likely, as I have discussed in this review, that the current that contributed *least* to the early records of i_{si} in multicellular preparations is $i_{Ca,f}$, which has nevertheless tended to acquire the label i_{si} in the single-cell work. This is likely to give rise to just as much confusion as was generated when the symbol i_{K2} was used (by Tsien and me) to describe the current that now turns out to *least* resemble the g_{K2} system originally described by Hall *et al.* (1963). Whether or not my suggested terminology is adopted, I think it is important to drop the non-specific label i_{si} when referring to the fast Ca current in single cells. The second reason why the confusion is unfortunate is that it is difficult to decide correctly what component(s) (if any) should be described as 'new'. Taking the admittedly very short history of the single-cell work as a basis, $i_{si,2}$ and $i_{Ca,s}$ are the 'new' currents, since they are not the already well-established fast current. But, of course, all of them must have been flowing across the cell membranes in the multicellular preparations (at some semantic level, no currents are 'new'!). The problem, as I have indicated above, is that it is not always easy to decide which component contributed to the total i_{si} in particular cases. What is certainly new is the much greater resolution provided by the single-cell-clamp work which makes it more plausible to dissect the components with some confidence. Currents that might have been dismissed as 'abominable notches' due to clamp escape at the surface membrane in multicellular preparations cannot be so dismissed in single cells when they carry a current only a small fraction of currents (like i_{Na} and the fast Ca current) which can clearly be clamped successfully.

Functions of the various components of i_{si}

Leaving these semantic problems aside, let us turn to function. How might the various components now be thought to contribute to the action potential and to rhythmic activity? I shall be discussing pace-maker activity later in this review. So far as the action potential is concerned, there are some very significant changes to be made to the picture given by the MNT and BR models. The fast component of i_{si} is sufficiently rapid (peak current is reached in 2–3 ms) that it would fuse in with the later part of the Na current peak. More important, its inactivation is so fast that it can hardly contribute much current (except in the form of a 'window' current) to the later parts of the action potential plateau. If we take the inactivation time constant to be 10–20 ms then inactivation would be expected to be largely over by 40–80 ms. The role of such a current in the plateau then comes to resemble more closely that of the Na current. Another important consequence of the increased speed is that the Ca ions required to trigger the contraction process are made available more quickly. If the Ca release from the sarcoplasmic reticulum is Ca induced then the speed of $i_{Ca,f}$ may be important in accounting for the speed at which $[Ca]_i$ has been observed to rise in experiments with aequorin (Allen & Kurihara, 1980; Wier, 1980). In attempting to reproduce these transients in our equations (which assume Ca-induced Ca ion release), DiFrancesco and I were greatly helped by the increased speed and intensity we were able to give to the Ca current. But the correlate of this speed for activation and inactivation of $i_{Ca,f}$ is that the maintenance of the plateau must depend on other components. As shown above (Fig. 9), $i_{Ca,s}$ is capable of maintaining a plateau, albeit lower than normal, in Cd-blocked cells. Together with $i_{si,2}$, it may well carry enough current to maintain the normal ventricular plateau in its later phases. Mitchell *et al.* (1983, 1984) and ter Keurs & Schouten (1984) give a similar role to the Na-sensitive current (? $i_{si,2}$) in rat ventricular cells. Hume & Giles (1983) also propose a late plateau role for the persistent inward current (? $i_{Ca,s}$) which they find in single frog atrial cells.

Finally, I should like to draw attention to a number of other interesting features of slow inward currents in the heart that have been described recently. Since they have been dealt with fairly fully in other recent reviews or papers I shall mention them only briefly with regard to their importance to work on reconstructing pace-maker activity in the heart.

Ca-entry-dependent inactivation

This phenomenon has been described in the rabbit s.a. node (Brown, Kimura & Noble, 1981; Brown, Kimura, Noble, Noble & Taupignon, 1984*a*), in frog atrium (Fischmeister & Vassort, 1981; Mentrard, Vassort & Fischmeister, 1983), in single frog atrial cells (Hume & Giles, 1982) and in Purkinje fibres (Marban & Tsien, 1981). These findings are, of course, important in relating work on the heart to the already well-established existence of this phenomenon in other cells. Is it of importance in normal cardiac electrical activity? I think the answer to that question must be yes. If the inactivation of $i_{Ca,f}$ were purely voltage dependent then it can be shown that the 'window' current that is necessarily generated by a Hodgkin–Huxley type of model is always too large and this results in a 'hump' of varying size during the

repolarization phase. In the s.a. mode modelling (Noble & Noble, 1984) we managed to keep this 'hump' to a minimum: it is only really evident on the plot of dV/dt (see Noble & Noble, 1984, Fig. 1). Nevertheless, the fact is that such a hump is not usually seen at all in normal s.a. nodal activity. We have subsequently modified the model to include Ca-dependent inactivation of $i_{Ca,f}$ and it is then relatively easy to eliminate the hump almost completely, particularly if the recovery from inactivation is assumed to take several hundred milliseconds, as shown experimentally in the s.a. node (Brown *et al.* 1984*a*) and in frog atrium (Mentrard *et al.* 1983).

The 'staircase' phenomenon

Another phenomenon that may be of importance in rhythmic activity is that during repetitive depolarizations i_{si} shows slow changes in amplitude that resemble the well-known tension 'staircases' in the heart (S. J. Noble & Shimoni, 1981*a*, *b*; Shimoni, 1981). Like the tension staircase, this sometimes leads to i_{si} *increasing* during repetitive depolarizations, whereas incomplete recovery would lead only to a decrease in amplitude. This phenomenon was first described in frog atrial trabeculae but it has also been observed in the rabbit s.a. node (Brown *et al.* 1984*a*).

This is a convenient point at which to comment on the correlation between i_{si} and tension changes in the heart. Broadly speaking, there are two possible schools of thought on this correlation. The first holds that it is by changing i_{si} and hence the quantity of Ca entering the cell, that the change in tension is brought about. This is the way in which adrenaline is thought to increase the force of contraction since it is well known that adrenaline increases i_{si} (Reuter, 1967; Reuter & Scholz, 1977). The alternative view is that changes in i_{si} *reflect* changes in $[Ca]_i$ that cause the changes in tension. This view is best exemplified by the accepted view of i_{TI} which is seen as being *induced by* the changes in $[Ca]_i$, not as causing those changes. But if, as on the interpretation I have given earlier in this review, i_{TI} is also activated (as $i_{si,2}$) during normal depolarizations to form a part of i_{si} then some changes, particularly those involving slow components of i_{si}, might be induced by $[Ca]_i$ changes rather than directly causing them. My suspicion is that some of the changes in i_{si} produced by Na–K pump inhibition (Lederer & Eisner, 1982) and by drugs like caffeine and tetracaine (see Fig. 1*C*) may be in this category. It is significant that these changes may run closely in parallel with the tension changes, rather than preceding them, as do the changes induced by catecholamines (see Lederer & Eisner, 1982).

But are the 'staircase' effects also $[Ca]_i$ induced rather than contributing to $[Ca]_i$ changes? This question is still open, but I presently favour the view that they may at least partly reflect rather than cause $[Ca]_i$ changes. There are two main lines of evidence for this view.

1. Using the s.a. node version of the DiFrancesco–Noble (1984) equations, Brown *et al.* (1984*a*) were able to reproduce the current 'staircase' by allowing changes in $[Ca]_i$ transients to induce changes in i_{NaCa}. These computations show, at least, that such a process could be *sufficient* to explain the phenomena.

2. Preliminary experiments on $i_{Ca,f}$ in single cells (E. Lee, K. S. Lee, D. Noble & A. J. Spindler, unpublished) have failed to show the 'staircase' effect. This would be compatible with the view that only the slower components, like $i_{si,2}$, are involved.

This fact must though be interpreted with care at this stage. It is important first to check that the tension 'staircase' really does exist in single cells since an alternative explanation for a failure to observe the current 'staircase' in single cells would be that intracellular Ca cycling may not be normal in isolated cells. A good test for this would be the existence of tension 'staircases'.

It is, of course, possible to develop a hybrid hypothesis: that some changes in i_{si} are Ca-induced *and* that they then contribute to $[Ca]_i$ changes (this would require that either $i_{Ca,f}$ or $i_{Ca,s}$ should be changed). This is the position favoured by Marban & Tsien (1982) in their work on cardiotonic steroids who point out that this would lead to the existence of a positive feed-back in the control of i_{si}. A small increase in the $[Ca]_i$ transient would increase i_{si} and (if this involves $i_{Ca,f}$ or $i_{Ca,s}$) in turn further increase the $[Ca]_i$ transient.

One possible difficulty with this view is that it remains to reconcile it with the view that $[Ca]_i$ *inactivates* $i_{Ca,f}$ (see previous section). Could $[Ca]_i$ both *inactivate* $i_{Ca,f}$ and *increase* the transient amplitude? Perhaps, but we really do not have any idea of the mechanisms that might be involved.

One of my reasons for introducing the influence of repetitive activity on i_{si}, or its components, is that any such effects may be important in analysing the contribution of i_{si} to the generation of repetitive activity. After all the heart never rests.

3. *Other current components*

The potassium currents, i_{K1} and i_K

By comparison with the developments concerning i_f/i_{K2} and i_{si}, the interpretation of the currents described as i_{K1} (the inward rectifier) and i_K (the delayed rectifier) has not changed so much. I shall therefore give only a brief treatment of the most important developments before proceeding to discuss the quantitative assessment of the contributions of these currents and of i_f and i_{si} to natural pace-maker activity in the heart.

The analysis of the inward rectifier has until recently tended to be relegated to a description of the current that remains when other (primarily time-dependent) currents have been analysed. This was the approach used in the development of the MNT model. But, this approach must incorporate the Na–K exchange pump current, i_p, and the steady-state value of the Na–Ca exchange current, i_{NaCa}, in the description of what functions in much the same way as the leak current, i_1, in the Hodgkin–Huxley model.

We can, however, now be more direct in approach. There are at least three lines of approach that have been used to characterize i_{K1} more specifically: (a) to use a blocker, such as Ba ions, to perform a subtraction of time-independent $i(E)$ relations in the presence and absence of the blocker (see DiFrancesco, 1981*b*); (b) to measure ^{42}K fluxes as a function of membrane potential (see Vereecke, Isenberg & Carmeliet, 1980), and (c) to use patch-clamp methods to study i_{K1} channels in isolation (Momose, Szabo & Giles, 1983; Sakmann, Noma & Trautwein, 1983). All these approaches confirm that i_{K1} is a strong inward rectifier and that it is very sensitive to $[K]_o$. This sensitivity to external concentration of K ions has two important consequences that are relevant to this review: first it allows large depletion current changes to occur during hyperpolarization. I have already discussed the significance of this fact in

relation to the reinterpretation of i_{K2}. Secondly, it means that any pace-maker system, such as the Purkinje fibre, that conducts i_{K1} fairly strongly, will be very sensitive to $[K]_o$, such that increasing $[K]_o$ will tend to abolish pace-maker activity. I have already referred to this property of Purkinje fibres in Fig. 4. These developments have largely served to confirm the properties of i_{K1} as assumed in previous work. There is however one development that introduces a significant difference. This is the demonstration that at very negative potentials i_{K1} rectification may show some time dependence. This was proposed by Carmeliet (1982) and has been confirmed recently in patch-clamp work on single channels (Kameyama, Kiyosue, Soejima & Noma, 1983). Moreover, Ba ion block of i_{K1} has been shown to be time dependent, which means that in the presence of Ba ions a form of pace-maker activity may develop in ventricular cells that is controlled by the time dependence of i_{K1} rather than of i_K or i_f (Carmeliet, Van der Heyden & Vereecke, 1983). This observation is important in relation to a later discussion in this review on s.a. node rhythm since the presence of nodal rhythm even in Ba-containing media has been used as an argument against the importance of i_K in s.a. nodal rhythm.

The delayed K current, i_K, has been studied in a number of cardiac preparations since its analysis in Purkinje fibres (McAllister & Noble, 1966; Noble & Tsien, 1969*a*). These include frog atrium (Brown, DiFrancesco, Noble & Noble, 1980), mammalian ventricle (McDonald & Trautwein, 1978), rabbit s.a. node (Katzung & Morgenstern, 1977; DiFrancesco, Noma & Trautwein, 1979), single frog atrial cells (Hume & Giles, 1983) and single sinus venosus cells from the frog (Shibata & Giles, 1983). The results all agree in showing that the voltage range of activation is from about -50 mV to 0 mV, that the channels show instantaneous rectification, but that they do not show the extreme sensitivity to $[K]_o$ displayed by i_{K1}. The cross-over phenomenon does not therefore occur so that a pace-maker mechanism strongly dependent on i_K should *not* necessarily be quiescent at high $[K]_o$. The s.a. node model based on the g_K decay hypothesis using i_K (Noble & Noble, 1984) does indeed show only a very moderate sensitivity to $[K^+]_o$ compared to that displayed by Purkinje fibres.

A comment on terminology is appropriate here. The first description of i_K used the symbol g_{K2} (Hall *et al.* 1963). This became confusing when the i_{K2} system emerged and Noble & Tsien (1969*a*) used the symbol i_{x1} in the belief that the delayed K current was a less pure K channel than i_{K2}. This view, of course, no longer carries any force since it is very probable that E_K is not after all so far away from the reversal potential for the delayed rectifier as was thought when the 'reversal potential' for i_{K2} was thought to be at E_K. I think the simplest way to resolve the terminological difficulties is to follow McDonald & Trautwein's (1978) use of the symbol i_K. This has been done in formulating the DiFranceso–Noble (1984) equations and I shall also adopt this terminology here.

The sodium current, i_{Na}

There have been very considerable advances made in the analysis of the Na current in the heart. This has in part resulted from better voltage clamping of multicellular preparations (e.g. Colatsky, 1980; Ebihara, Shigeto, Lieberman & Johnson, 1980), in part from work on isolated single cells using whole cell clamps (Lee, Weeks, Kao, Akaike & Brown, 1979; Brown, Lee & Powell, 1981; Lee, Hume, Giles & Brown, 1981; Bodewei, Hering, Lemke, Rosenshtraukh, Undrovinas & Wollenberger, 1982) and

using patches (Cachelin, De Peyer, Kokubun & Reuter, 1983). The kinetics determined show considerable quantitative differences from those used in the MNT model and much of the new data was used in formulating new equations for this current in the DiFrancesco–Noble (1984) equations. This formulation gives a good reconstruction of the steady–state ('window') current (Attwell, Cohen, Eisner, Ohba & Ojeda, 1979), which has been shown to contribute to the maintenance of the plateau in Purkinje fibres and which may be involved in low voltage pace-maker activity in these cells.

The sodium pump current, i_p

It has been clear since Isenberg & Trautwein's (1974) work that a significant outward current may be carried in the heart by the Na–K exchange pump. In a very elegant series of experiments Gadsby (1980), Eisner & Lederer (1980) and Daut & Rüdel (1982) have succeeded in characterizing this current sufficiently well for a quantitative formulation to be possible for the first time. This has been incorporated into the DiFrancesco–Noble equations. The Na–K pump current has also been detected recently in isolated frog atrial cells (Shibata, Momose & Giles, 1983).

4. *The natural pace-maker: the s.a. node*

I have already discussed the fact that normal pace-maker activity in Purkinje fibres does *not* conform to the g_K decay hypothesis. What about the natural pace-maker, the s.a. node?

Alternatives to the g_K decay hypothesis

Until about 1976 the s.a. node itself was thought to be too difficult for voltage-clamp experiments. The best available evidence therefore came from analogies with partially depolarized Purkinje fibres (Hauswirth *et al.* 1969) or atrial trabeculae (Brown, Clark & Noble, 1972, 1976). In both cases, the suggested mechanism was a decay of K current transported by the delayed rectifier followed by activation of the second inward current, i.e. a version of the g_K decay hypothesis. This hypothesis was fully supported by the Brown *et al.* (1977) study of the frog sinus venosus. Although they found i_f in this tissue, they also pointed out that its activation range was usually too negative for it to be involved in generating the pace-maker depolarization. Yet the threshold for activation of i_{si} was situated towards the last 30–50% of the pace-maker depolarization. These observations virtually eliminated i_f and i_{si} as the primary cause of the pace-maker depolarization, leaving i_K decay as the only remaining viable hypothesis. Since then, the main object of study has been the mammalian s.a. node, and in particular the small dissected rabbit s.a. node preparation pioneered by Noma & Irisawa (1976*a*). Four theories have emerged:

1. Noma & Irisawa and their colleagues (see e.g. Yanagihara & Irisawa, 1980) have proposed that the main pace-maker current is i_{si} and that i_K decay or i_f onset are not important or are very small compared to the changes in i_{si}.

2. Maylie, Morad & Weiss (1981) also proposed that i_K decay was not important but that i_f onset was involved.

3. Pollack (1976) has proposed that the rhythm depends on intercellular mechanical interactions that are implicated in propagation in nodal tissues.

4. Brown *et al.* (1982) have proposed that the original g_K decay hypothesis is correct.

The field is now somewhat confused (or, at least, confusing to the uninitiated) because some of these authors have explicitly or implicitly abandoned their original views and I suspect that there may soon be a degree of unanimity on some form of the g_K decay hypothesis. Regardless of authorship, or ownership, though, the arguments for the alternatives are interesting in their own right and it is partly because I think that it is instructive to weigh them up that, in this review, I have chosen to discuss the four theories in turn.

First, then, the i_{si} hypothesis. There is, of course, no denying the fact that i_{si} (but which components?) is of crucial importance to s.a. node activity. S.a. node pace-maker activity is not abolished by TTX and does not therefore require i_{Na}. But, to take an analogous situation, the fact that the upstroke in Purkinje fibres is generated by i_{Na} does not lead to its being identified as the 'pace-maker' current in this tissue; so the fact that the upstroke is generated by i_{si} in the s.a. node does not identify it as the 'pace-maker' current. The crucial question is not what generates the upstroke but what brings the membrane potential towards the threshold for generating the upstroke. Now this *could* be the same mechanism as that which generates the upstroke provided that its 'threshold' is very broadly spread over the voltage range of the pace-maker depolarization. Voltage-dependent conductances of the Hodgkin–Huxley type do not of course have a strictly defined threshold. What is meant here is the spread on the voltage axis of the foot of the activation curve. Thus, in the case of the Na conductance this is sufficient to allow i_{Na} to contribute a significant fraction of the depolarizing current to the last 30% or so of the pace-maker depolarization in Purkinje fibres (this is a feature both of the MNT (1975) and the DiFrancesco–Noble (1984) equations). But the extent of the foot is not large enough to enable i_{Na} to *initiate* the pace-maker depolarization. Rather, it increasingly helps the depolarization, once it is already well developed, to succeed in developing spontaneously into a fully-fledged action potential.

I think the experimental evidence suggests a similar role for i_{si} in the case of the s.a. node pace-maker depolarization. Measurements of the threshold for i_{si}, both in frog sinus venosus (Brown *et al.* 1977, Fig. 13) and in the rabbit s.a. node (Brown *et al.* 1984*a*) show that it is not significant at the beginning of the depolarization and that it is largely during the last third of the depolarization that i_{si} becomes significant. The question which component(s) of i_{si} are then significant is hard to answer with certainty. The inward current that dominates the record when a voltage clamp is imposed at the end of the pace-maker depolarization activates very slowly indeed and is i_{TI}-like in its time course (this is the component I have earlier described as $i_{si,2}$, see Fig. 7). But, of course, if $i_{Ca,f}$ is also activated it would already be so, and be close to its steady-state value, in such an experiment since the rate of change of voltage during the pace-maker depolarization is so slow compared to what we now know are the very fast kinetics of activation of $i_{Ca,f}$.

How then did Noma and Irisawa and their colleagues come to the view that i_{si} is more important than this analysis suggests? I think the answer to that lies in an analysis of the dynamics of i_K during a pace-maker depolarization. Their measurements of the kinetic properties of i_K are similar to those of the Oxford group (and to those published by DiFrancesco *et al.* (1979) – I shall show later that this *requires* i_K decay to be the main initiator of the pace-maker depolarization). But, in their

computations of pace-maker activity using these kinetics they found that i_K remains fairly constant during the pace-maker depolarization (see Yanagihara, Noma & Irisawa, 1980). How is this consistent with the g_K decay hypothesis? To answer this question it is important to note that it is g_K not i_K that matters. To clarify this point consider the following argument. The rate of change of potential during a pace-maker depolarization is small and nearly constant so that we can, to a first approximation, regard the net ionic current, which will be $-C\,\mathrm{d}V/\mathrm{d}t$ (where C is the membrane capacity), as nearly constant. Suppose, for the sake of argument that the background inward current, $i_{b,in}$ does not vary greatly over the pace-maker range of potentials so that this may also be regarded as nearly constant. Then, approximately,

$$i_K = -i_{b,in} - C\,dV/\mathrm{d}t \simeq \text{constant}. \tag{4}$$

Thus i_K will be nearly constant even if g_K is varying in precisely the way required to be the prime cause of the change in potential. This argument was illustrated graphically in Brown *et al.* (1977, Fig. 12). Thus, if g_K is decaying, the depolarization must increase the driving force sufficiently for i_K itself to remain nearly constant. What is certain (since the instanteous $i(E)$ relation for i_K does not show a negative slope region) is that i_K will change by much less than g_K does. I will return to a more quantitative version of this argument when I have considered the other theories.

Maylie *et al.* (1981) used a potentially powerful and ingenious approach based on measuring $[K]_0$ changes with an ion-sensitive electrode. Their argument is that the rate of change of [K], $\mathrm{d}[K]/\mathrm{d}t$, should reflect, in part at least, the K flux across the membrane. If a large part of the current relaxation that occurs when one uses a voltage clamp to stop the pace-maker depolarization is due to i_K decay then the rate of decay of [K] should initially be slow and should then increase as i_K decays. But their experimental results showed that, even when the total current relaxation is large $\mathrm{d}[K]/\mathrm{d}t$ is nearly constant. Their conclusion was that i_K decay is not important and that the majority of the current relaxation must be attributable to the onset of i_f (which they call i_p).

If the conditions of the experiment were ideal, i.e. if the extracellular K electrode was sensing an undisturbed [K] deep in the restricted spaces of the preparation it would be difficult to find fault with this argument. And, indeed, I was myself initially very puzzled by this result since, whatever its quantitative role in pace-maker activity, g_K does switch on significantly during each action potential and must decay again afterwards. When developing the DiFrancesco–Noble (1984) equations into a version for the s.a. node (Noble & Noble, 1984) we therefore made use of the fact that the model represents non-uniform distribution of K ions in the extracellular space with appropriate partial differential equations. Our computations (see Brown *et al.* 1984*b*, Fig. 4) confirm that the argument of Maylie *et al.* (1981) would indeed be valid for an undisturbed space but they also show that it would not be valid for a near surface or for a disturbed space. In such cases it is then quite easy to compute a relatively constant $\mathrm{d}[K]/\mathrm{d}t$ even when i_K varies substantially.

Very recently, Noma, Morad & Irisawa (1983) have published a paper using Ca block of i_f to show that it plays only a minor role in the s.a. node pace-maker potential, though they still give the most important role to i_{si} (see my review of this view above).

The third theory mentioned above is that of Pollack who argues that intercellular

mechanical forces are important in pace-maker activity. Part of the basis for this view is that intercellular electrical connexions (nexuses) are fairly rare in s.a. node tissue and that conduction may itself require other forms of intercellular interaction (see Pollack, 1976). There are two main arguments against this line of approach. Perhaps

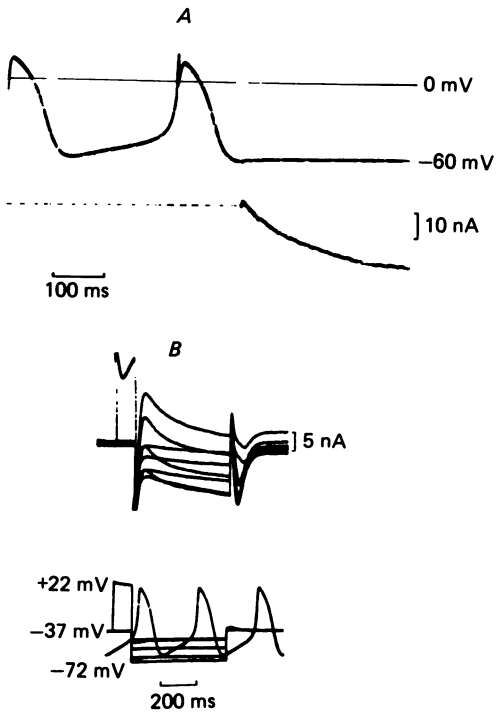

Fig. 10. Estimation of relative contributions of i_K and i_f to the pace-maker depolarization in rabbit s.a. node. *A*, this shows the current relaxation recorded when the voltage clamp is imposed at the maximum diastolic potential (in this case −60 mV). About 20 nA of current relaxation in the inward direction occurs during the period of time (about 200 ms) taken up by the pace-maker depolarization. *B*, this shows how the likely relative contributions of i_K decay and i_f onset to the current relaxation can be estimated. First, a depolarizing pulse (in this case to +22 mV for 100 ms) is found that activates a current relaxation very similar to that activated by an action potential. Not surprisingly, this requires a square pulse similar in amplitude and duration to the action potential. The current relaxations at various potentials produced by this pulse are then compared with those produced by simple hyperpolarization from a potential (in this case −37 mV) chosen to be at the foot of the i_K activation curve (so that no i_K decay contributes to the relaxations). This voltage is also positive to the foot of the i_f activation curve. Subtraction of the two relaxations at each potential then gives an estimate of the contribution of i_K decay. It can be seen that, at all potentials within the pace-maker range, i_K decay is much larger than i_f onset. Only when E_K is approached (towards −80 mV) does i_f decay dominate. (Brown *et al.* 1984*b*.)

the most powerful is that isolated single cells show normal pace-maker activity. Shibata & Giles (1983) have analysed the ionic currents underlying pace-maker activity in isolated frog sinus venosus cells and their work not only shows that rhythmic activity occurs in single node cells but also that it conforms to the g_K decay hypothesis, with i_{si} playing a role towards the end of the pace-maker depolarization.

Isolated mammalian s.a. node cells also show perfectly normal rhythmic activity (Irisawa, Nakayama, Kurachi & Noma, 1983). The other line of argument is a theoretical one. Astonishingly, few nexus channels are required to allow synchronization of pacing cells (de Haan, 1982). In fact, a *single* nexus channel per cell could be sufficient (see Noble, 1982*a*). This density would be even lower than that resolvable by electron microscopical techniques!

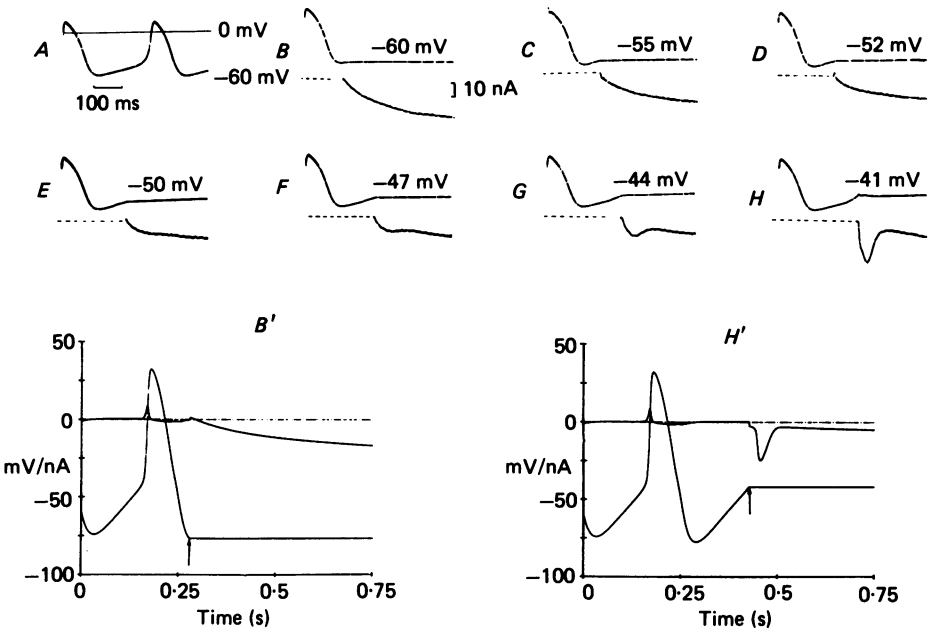

Fig. 11. Progressive change in current relaxations as the s.a. node pace-maker potential develops. The first change is that the current changes due to i_K decay and i_f onset (see Fig. 10) become smaller. This happens at about the speed expected for the i_K decay process, whose time constant varies only slightly over this narrow range of potentials. Then, beginning at about −50 mV the relaxation speeds up again and by −47 mV it is quite clear that an additional inward transient is superimposed on the remaining decay of i_K. This transient becomes larger and faster during the last few millivolts before the action potential upstroke occurs. Records *B'* and *H'* show computer reconstructions of traces similar to those shown experimentally in *B* and *H*. The slow transient inward current here is the component we have called $i_{si,2}$. See text for discussion of presence and role of $i_{Ca,f}$. (From Brown *et al.* 1984*b*.)

Recent evidence for the g_K decay hypothesis

It will already be evident from this discussion that I favour the g_K hypothesis in the case of the s.a. node, despite having abandoned it in the case of the Purkinje fibre. The time has come to review carefully the evidence in favour of this view. Some of this evidence has already been discussed in rejecting the alternative views. But there remains a body of evidence which is more directly aimed at substantiating the hypothesis. This has been described recently in Brown *et al.* (1982) and Brown *et al.* (1984*b*).

A key feature of the work described in these papers is to perturb the preparation and its normal pace-maker activity as little as possible. Natural pace-maker activity is allowed to develop and the voltage clamp is applied at various times during the depolarization. The results are then supplemented with the results of more

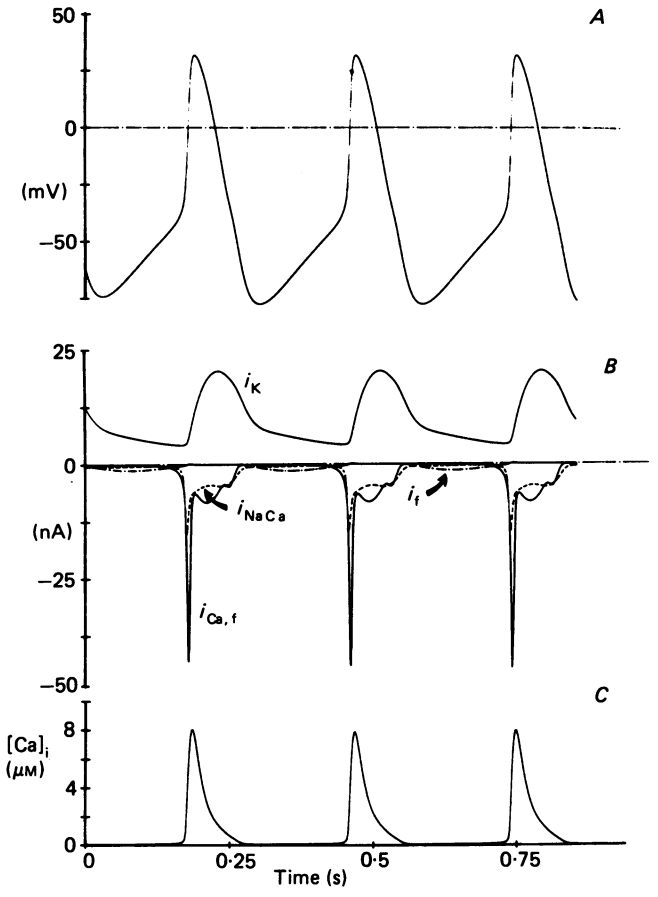

Fig. 12. Computed records summarizing our present picture of normal rhythmic activity in the s.a. node. *A*, computed variation in membrane potential. *B*, computed variations in $i_{Ca,f}$, $i_{si,2}$ (reconstructed in the model as the Na–Ca exchanger current, i_{NaCa}), i_K and i_f. These results resemble those computed by Noma & Irisawa's group, except for the presence of $i_{si,2}$, which is not represented in their model. *C*, computed variations in $[Ca]_i$. (Based on Brown *et al.* 1984*b*.)

conventional voltage-clamp pulse protocols. This approach is not new. It was pioneered by Vassalle (1966) in his work on the Purkinje fibre pace-maker depolarization. The advantage of this protocol is that it enables quantitative assessments to be made of the magnitudes of the various current components flowing at various times during the pace-maker depolarization. The disadvantage is that these currents are very small, as indeed they must be given the very small rate of change of potential during the pace-maker potential.

Fig. 10 shows the result of such an experiment on the rabbit s.a. node. It is clear that when the clamp is imposed at the beginning of the pace-maker depolarization there is a current relaxation that could be either decay of i_K or onset of i_f. Fig. 10*B* shows how the relative contributions can be assessed. First, the record at the beginning of the pace-maker depolarization was reproduced by recording the current

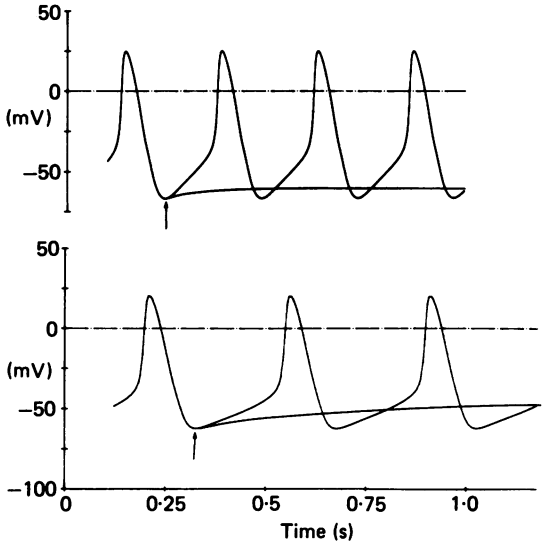

Fig. 13. Quantitative demonstration of the essential role of g_K decay in generation of s.a. node pace-maker potential. The top records show the behaviour of our model, while the lower records show the behaviour of the 'Japanese' model when the decay of the gating variable controlling i_K is stopped at the time of the maximum diastolic potential. The two models then agree in showing only a very small pace-maker depolarization which is attributable to the small influence of i_f.

following a short depolarizing pulse that activates a similar current relaxation as did the naturally occurring action potential. A second voltage-clamp trace is then obtained by omitting the depolarizing pulse. The key feature of this experiment is that the initial holding potential is chosen to be at the bottom of the i_K activation curve. Thus, no i_K will be present in the second trace and any remaining current relaxation may be attributed to i_f. Clearly, i_K decay forms by far the largest component of the total current relaxation. This approach may be extended to determine how quickly i_K decay occurs during the pace-maker depolarization. It is clear from Fig. 11 that when the pace-maker potential has reached the last third or so of its time course the current recorded ceases to be dominated by a monotonic relaxation and is increasingly dominated by a transient inward current whose time course identifies it as $i_{si,2}$. It would be tempting from this to conclude that the component of i_{si} involved is a very slow component, such as would be produced by i_{NaCa}. And, indeed, the model results, which incorporate a description of i_{NaCa}, do reproduce the very slow current (see Fig. 11). However, if the model is a good reconstruction then this tempting conclusion would be incorrect. The component of i_{si} that is involved in the last third of the pace-maker depolarization in the model is the fast component, which I have called $i_{Ca,f}$. The reason it is not seen either in

the experimental or model results is that, at each voltage, it is already activated. On this time scale, the speed of the fast component of i_{si} ensures that it is almost at its steady-state level at each voltage. There are two further reasons for thinking that the i_{TI}-like component of i_{si} is not crucial for pace-making. First, if it is induced by the $[Ca]_i$ transient that produces contraction then contraction would begin during

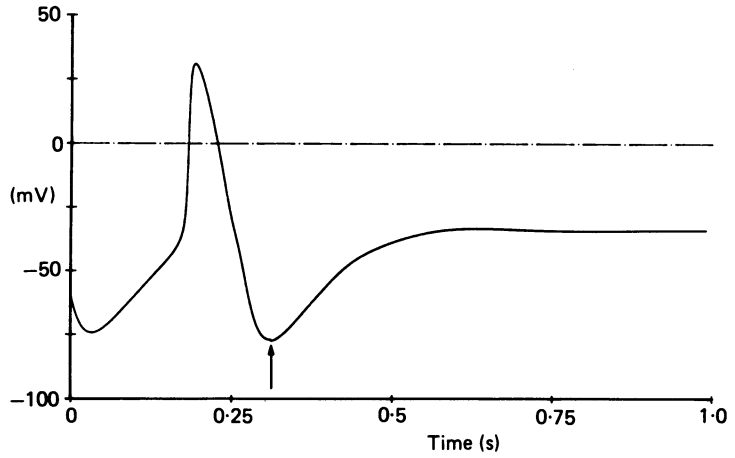

Fig. 14. This shows the complementary calculation to that shown in Fig. 13. Here, instead of stopping the i_K decay, we blocked the Ca current $i_{Ca,f}$. It is clear that the first two-thirds or so of the pace-maker depolarization is unaffected by this change. I have not shown the equivalent behaviour of the Yanagihara *et al.* (1980) model since, as the authors note (see their p. 854) the model fails to reproduce the effect of Ca current block. The difference lies in the fact that the 'Oxford' model distinguishes between the D-600-sensitive Ca current and the background inward currents, whereas the 'Japanese' model does not. This difference does though serve to emphasize the importance of background inward current in pace-maker activity in the heart. These background channels remain the least characterized of the current components.

the pace-maker depolarization. This is not the case. The second reason is that the results shown in Fig. 10 are not found in all experiments. In some cases, the i_{TI}-like component is not evident until one clamps at a time and voltage which would more properly be counted as part of the upstroke of the action potential. I think this reflects natural variation in the membrane depolarization or the level of $[Ca]_i$ at which the regenerative release of Ca (which is what in turn generates i_{NaCa} in the model) occurs.

These findings are summarized in Fig. 12 which shows our present interpretation of the variations in $i_{Ca,f}$, i_K, i_f and $i_{si,2}$ (here attributed in the model to i_{NaCa}) during natural pace-maker activity in the s.a. node.

There are two further uses to which the model may be put that are relevant to testing the g_K decay hypothesis.

Fig. 13 shows the result of 'freezing' the i_K gating process at the time of the maximum diastolic potential. It is clear that this abolishes pace-maker activity. The small depolarization that remains illustrates the small calculated contribution of i_f to the pace-maker potential. In this Figure I have shown the result of this computation both on the s.a. node model based on the DiFrancesco–Noble (1984)

equations and on the model developed by Yanagihara *et al.* (1980). It is clear that the results are essentially identical, as indeed they should be since the kinetic description of i_K is fairly similar in the two models.

The second use of the model is shown in Fig. 14. This shows the result of blocking the fast Ca current, as would occur in the presence of D-600. It can be seen that the great majority of the pace-maker depolarization still occurs and the membrane settles down at a fairly depolarized level (around −35 mV). This is also what is found experimentally.

Contribution of i_f to natural pace-maker activity

Can we produce more direct evidence for the small contribution made by i_f? This has been done experimentally by using Cs ions which at low concentrations (1 mM) is a fairly selective blocker of i_f. This usually slows the pace-maker rhythm by about 10–20% (Brown *et al.* 1982; Brown *et al.* 1984*b*) which corresponds well to the expected contribution based on the model computations. Noma, Morad & Irisawa (1983) have also recently described the use of Cs in a paper in which they propose that i_f is not involved at all. Their experimental results though show a small slowing (see their figs. 1 and 5) of the same order of magnitude as in our experiments.

The reason I have emphasized this small role of i_f is that it is still an open question whether i_f might not play a larger role in some circumstances. For example, it is increased by adrenaline and it may be that some part of the acceleration induced by adrenaline may be attributable to i_f, particularly since the s.a. node is not at all uniform in its electrophysiology (see review by Brown, 1982). Shifts of the dominant pace-maker region are known to occur in adrenaline, possibly to cells in which i_f makes more contribution. In this connexion it is interesting to note that adrenaline accelerates the depolarization *throughout* the duration of the pace-maker potential (Hutter & Trautwein, 1956). If it acted only by increasing i_{si} one would expect the acceleration to occur only during the second half of the pace-maker potential.

i_{K1} in the s.a. node

I have dealt with the role of i_K, i_f and i_{si} in s.a. node pace-maker activity. What about i_{K1}? The significant feature here is that it is nearly absent. This has very important consequences since it is the sensitivity of i_{K1} to $[K]_o$ that allows very moderate changes in $[K]_o$ to modulate greatly the frequency of the pace-maker rhythm in Purkinje fibres. By contrast the s.a. node rhythm is much less sensitive to $[K]_o$. This lack of sensitivity is well reproduced by the model equations (see Noble & Noble, 1984, Fig. 3).

5. *Abnormal rhythms and the possible roles of i_{NaCa} or i_{NaK}*

It will already be clear from this review that, in clear contrast to the situation only a few years ago, there is considerable diversity in the pace-maker mechanisms of the heart. So far, I have reviewed three mechanisms: g_K decay (in the s.a. node), i_f –mainly Na current – onset (in normal Purkinje fibre rhythm) and time-dependent changes in i_{K1} (ventricular rhythm induced by Ba ions). These mechanisms do nevertheless have one important feature in common: they all depend on surface membrane voltage-induced current relaxations. Hence, the underlying oscillations of membrane

current are completely suppressed in voltage-clamp conditions. The fourth mechanism does *not* share this feature. This is the rhythm generated when the Na–K pump is blocked. Lederer & Tsien (1976) showed that this rhythm depends on an inward current, i_{TI}, that is not identified with variations in voltage-dependent currents normally implicated in cardiac rhythm. Moreover, oscillations in this current can continue even in voltage-clamp conditions and have therefore been attributed to current changes induced by intracellular oscillations in [Ca] (see review by Tsien, Kass & Weingart, 1979). My colleagues and I have recently investigated a possible theoretical model for such oscillation based on three main assumptions.

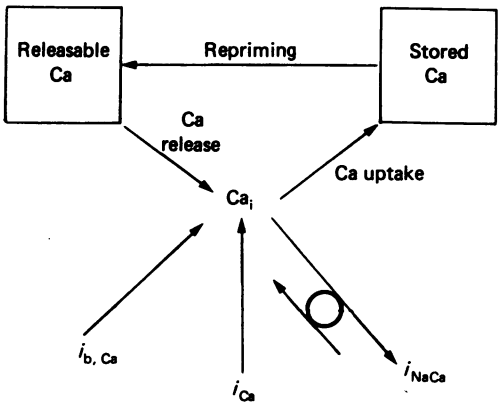

Fig. 15. Diagram summarizing the processes assumed in the DiFrancesco–Noble model to control Ca ion movements. An energy-consuming pump is assumed to transport Ca into the sarcoplasmic reticulum which then reprimes a release store. Release is activated by cytoplasmic free Ca (Ca_i). Ca leaves the cell via the Na–Ca exchanger and may enter the cell through the voltage-gated Ca channel i_{Ca} or through a background (leak) channel $i_{b,Ca}$. Rarely, it may enter the cell through the exchanger (when $[Ca]_i$ is very low and the voltage very positive). These were the minimum assumptions necessary to model the $[Ca]_i$ transient and to model oscillatory variations at raised $[Ca]_i$. There are though some important Ca movements that are neglected in this simple model, such as binding to contractile proteins, to calmodulin, buffering by mitochondria, and transport via a surface ATP-driven pump. The purpose of the model is restricted to demonstrating some minimal assumptions required to model the behaviour of $[Ca]_i$ for electrophysiological purposes.

1. That Ca ion release from the sarcoplasmic reticulum is induced by Ca ions (the Ca-induced Ca release hypothesis of Fabiato & Fabiato, 1975).

2. That, following release, the release store is slowly reprimed by a voltage-dependent process (this idea was first proposed for skeletal muscle by Hodgkin & Horowicz, 1960 and Gibbons & Fozzard (1975) have described very similar results in cardiac muscle).

3. That variations in $[Ca]_i$ induce variations in the current carried by the Na–Ca exchange mechanism. For this we have used a model based on that of Mullins (1977, 1981) – see DiFranceso & Noble (1984).

These main assumptions are illustrated diagrammatically in Fig. 15. When incorporated into a model of cardiac electrical activity that represents the other membrane currents, it is possible to show that Na–K pump inhibition is then a sufficient condition to induce oscillations even under voltage-clamp conditions. An

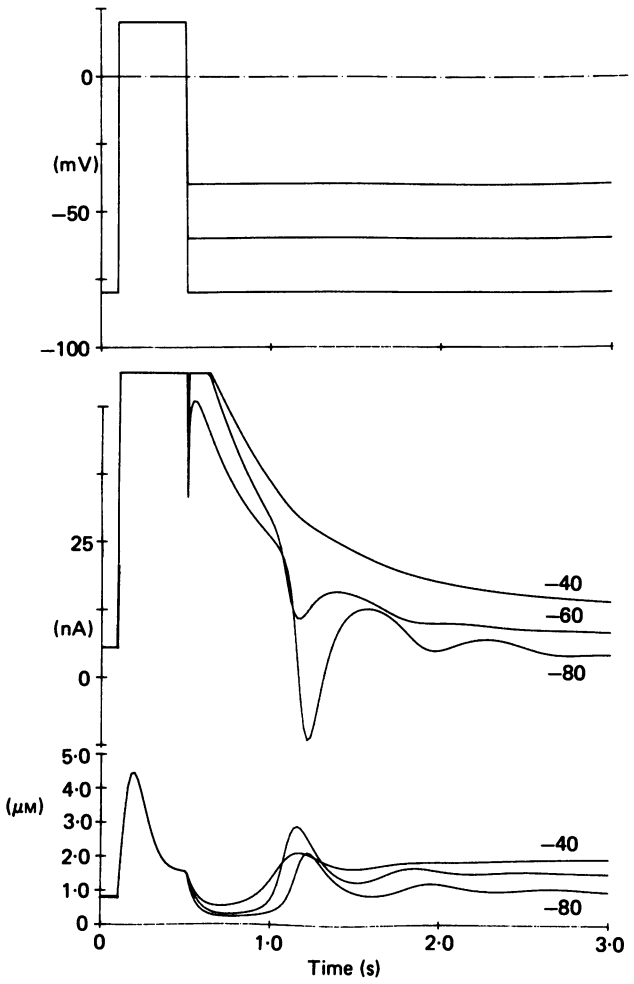

Fig. 16. Use of the DiFrancesco–Noble equations to reproduce oscillatory current changes and variations in $[Ca]_i$ when the Na–K exchange pump is partially blocked. In this particular set of computations the pump was reduced to 25 % of its normal capacity, which allows $[Na]_i$ to rise from its normal value of about 8 mM to about 20 mM. This is a sufficient condition for the generation of oscillatory current changes. Top: voltage-clamp steps. Middle: computed net membrane currents. Superimposed on the onset of i_f and decay of i_K (which are responsible for the general downward current relaxation at the end of each step) there is an inward current whose onset and decay are almost symmetric (cf. Fig. 1*C*), followed by a damped oscillation. The peak amplitude is voltage dependent and the steepness of this dependence on voltage depends on the function chosen for the voltage dependence of the Na–Ca exchanger. In this case a steep sinh function (cf. Mullins, 1981) was used and this reproduces the kind of experiment (cf. Hennings & Vereecke, 1983) in which i_{TI} falls towards zero at about −40 mV. A small phase shift also occurs (cf. Brown *et al.* 1984). Bottom: computed variations on $[Ca]_i$. Note that, before the oscillatory variations, the Ca concentration falls below the steady-state level. (D. DiFrancesco, G. Hart & D. Noble, in preparation; see DiFrancesco, Hart & Noble, 1983.)

example (from D. DiFrancesco, G. Hart & D. Noble, in preparation) is shown in Fig. 16. There are several features of this result that are worth noting. First, as in the experimental recordings of i_{TI} (or of $i_{si,2}$ – see previous sections of this review) the onset and decay of the inward current are almost symmetric. Secondly, they are generated by oscillations in $[Ca]_i$ that occur following repolarization. These oscillations (like those recently described experimentally using aequorin – see Allen, Eisner, Lab & Orchard, 1983) lead to Ca levels *below* those achieved in the steady state as well as levels above the steady-state value. Thirdly, the frequency lies in the range 1–2 Hz, as also seen experimentally in Na-pump-blocked Purkinje fibres. In the model this frequency depends on the repriming time constants for transfer of Ca to the releasable state. We used figures similar to those obtained experimentally by Gibbons & Fozzard (1975).

I should emphasize that there may be nothing very unique about the particular model used in these equations with respect to these results. Other models for the current involved (e.g. allowing $[Ca]_i$ to activate the non-specific channels, i_{NaK}, rather than the exchange current, i_{NaCa}) might work equally well, though there would be some differences since i_{NaCa} *contributes* to variations in $[Ca]_i$ as well as being activated by them, whereas the non-specific current would not contribute directly to variations in $[Ca]_i$. Furthermore, other models for the time dependence of the release process and its repriming are perfectly plausible. Further clarification of these abnormal rhythm mechanisms will depend on obtaining further precise evidence on the composition of i_{TI} and on the mechanisms of internal calcium release.

Conclusions

It will be clear from this review that anyone who hoped that the introduction of single-cell and patch-clamp methods would help to reduce the number of separate ionic current mechanisms in the heart will have been disappointed. On the contrary, the number has further increased. Nor is this phenomenon restricted to the heart. In a recent review I discussed the increasing number of strong parallels to be found between many of the old and new cardiac mechanisms and those being found in nerve cells (Noble, 1983).

It is interesting to compare the number of separate mechanisms postulated in the 1962, 1975 and 1984 Purkinje fibre models. I have done this in Table 1.

The horizontal and vertical lines and organization of this Table are all significant. Horizontal lines are used to classify the current mechanisms according to the types listed at the left, while vertical lines are used to indicate where reinterpretations have taken place. This allows mechanisms that have been reinterpreted to be classified both under their original category and in their new category. At the right I have listed the main activators and examples of known blockers.

It is sincerely to be hoped that this is not a case of indefinite exponential growth or, by the year 2000, we shall all have great difficulty in explaining cardiac excitation to ourselves, let alone to students with formidable memories!

Can we really be sure that so many separate components exist? It is worth noting that, of the mechanisms that could appear as current jumps in patch-clamp studies (clearly, pump and exchange mechanisms are well beyond resolution at single-current jump level), the majority have already been identified in such studies. These include

TABLE 1. Summary of ionic current mechanisms in the heart

	Ionic current mechanisms in heart				
	1962	1975	1984	Activator	Blocker
Na current	i_{Na}	i_{Na}	i_{Na}	Depol.	TTX
K currents	i_{K1}	i_{K1}	i_{K1}	Hyperpol.	Ba
	i_{K2}	i_x	i_K	Depol.	Ba
Cl current		i_{qr}	i_{to}	$[Ca]_i$ depol.	4-AP
		i_{K2}	i_f	$[K]_o$ hyperpol.	Cs
Non-specific currents			i_{NaK} ($?i_{TI}$)	$[Ca]_i$	?
Ca currents		i_{si}	$i_{Ca,f}$	Depol. (Ca inact)	Cd D-600
			$i_{Ca,s}$	Depol.	?
			$i_{si,2}$ ($?i_{TI}$)	$[Ca]_i$	?
Exchange and pump currents			i_{NaCa}	$[Ca]_i$ $[Na]_o$	?
			i_p	$[K]_o$ $[Na]_i$	Ouabain
Passive background currents	i_b	$i_{b,in}$	$i_{b,Na}$		
			$i_{b,Ca}$		
	i_{Cl}		i_{Cl}		

The Table lists the current components assumed in the 1962, 1975 and 1984 Purkinje fibre models and classifies them according to their ionic composition. Where this has been reinterpreted, a vertical line is used. Where the terminology has changed, e.g. from i_{K2} to i_x to i_K, this is indicated by arranging the relevant terms on the same horizontal row. Even where the terminology has not changed though (e.g. as with i_{K1}) there may have been significant changes in the formulation of the equations for the component in the different models. The main uncertainty in this Table now lies in the area between the Ca currents, the exchange pump current and the Ca-activated non-specific current. Our interpretation of the second component of i_{si} ($i_{si,2}$) is that it may be the same mechanism as the transient inward current, i_{TI} and that both are to a large extent carried by the Na–Ca exchanger as i_{NaCa}, but another possibility is that these components are carried by the non-specific current, i_{NaK}, activated by intracellular Ca. i_{TI} is therefore included in the Table together with a query in both the appropriate places.

to my knowledge: i_{Na}, i_{K1}, i_K, i_{to} (probably – at least Ca-activated K currents have been seen), i_{NaK}, $i_{Ca,f}$. Of the components that remain: i_p has been seen in single cells; $i_{Ca,s}$ has also been seen (indeed, identified as such) in single cells, but may be very difficult to find in patch studies of single channels since either the density of channels is much lower (only about 20 %) than for $i_{Ca,f}$ or the single channel current is much smaller. Thus we are left only with the background currents and $i_{si,2}/i_{NaCa}$. Could we, at least, eliminate these?

In theoretical work with DiFrancesco and Hart, I have tried very hard to do so. For, when Colqhoun *et al.* (1981) identified their Ca-activated non-specific channels in patch-clamp studies it became very tempting to conclude that here, at last, were the background current channels. And, of course, if they were significantly activated even at resting levels of $[Ca]_i$, then *a fortiori* they would be activated during $[Ca]_i$ transients so there would be no need to postulate a separate mechanism for $i_{si,2}$ and indeed no particular need to include the exchanger current, i_{NaCa}.

The opposite position has been very forcefully expounded by Mullins in his recent book *Ion Transport in Heart* (Mullins, 1981) where i_{NaCa} (called i_c by Mullins) has been proposed as responsible for a wide variety of already identified current components in the heart. I have already expressed my reasons for doubting some of Mullins' conclusions (see Noble, 1982*b*). But, on his essentially important conclusion, that the exchanger current may contribute to the second inward current, I think I must agree. My reasons are primarily theoretical, but they are as well researched as were the reasons in the early 1960s for being convinced, on theoretical grounds, that i_K (or something like it) must exist (I have discussed this point at the beginning of this article). They also illustrate what I see to be one of the main purposes of theoretical models in this field: to test the *quantitative* plausibility of various hypotheses.

And it is on grounds of quantitative implausibility that I find it very difficult to give a major role to the non-specific current, i_{NaK}, in normal electrical activity. I have reviewed the arguments in earlier sections of this review and they lead me to conclude that, if we are to reduce Table 1 at all it will be by identifying $i_{si,2}$ as largely i_{NaCa}. We could then make the Table much neater by closing the uncertain gap between Ca currents and exchange currents. This, or some alternative rationalization of the problems I have discussed here, must be one of the major aims of single cell work in the immediate future.

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Note added in proof. There are, of course, surprising and exciting developments that could not be covered in this review. One of the most important of these (the recording of single channels) has been dealt with in a valuable review by Reuter (*A. Rev. Physiol.* 1984, in the Press).

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