Calcium Balance in the Heart

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It is well established that cardiac contraction is initiated by calcium entering the cells via calcium channels and that this triggers release of calcium from the sarcoplasmic reticulm. But how does calcium leave the cell? During systole or during diastole? By what mechanism? And is this mechanism electrogenic? If so, does it affect the action potential? These are the questions addressed in this lecture.

As shown in Figure 1 (a) the classical idea was that calcium enters the cells throughout the duration of the action potential and that this is ensured by the very long duration of the action potential plateau and very slow inactivation of the calcium channels. Calcium is then pumped out (perhaps by the sarcolemmal calcium pump) during the interval between action potentials. The idea that I shall develop in this lecture is very different. It is that calcium entry occurs primarily during the early phase of the action potential and that calcium exit occurs during the later phase of repolarization, leaving very little calcium efflux to occur during diastole. This idea is illustrated in Figure 1 (b).

The classical idea was based on the fact that the calcium current was thought to be

activated and inactivated very slowly compared to the sodium current. The exact time course of what was then called the 'slow inward current' (or i_{si}) varied from tissue to tissue but the time for activation varied from about 5 msec to about 50 msec, while the inactivation time varied from about 100 msec to several seconds (for a review see Noble, 1984). These figures were obtained from voltage clamp experiments performed on multicellular preparations and we now know that there are many complications in the interpretation of this work. In particular, the time course of activation is slowed partly by series resistance in the extracellular spaces and partly by overlay with the transient outward current, while part of the slow inactivation may have been due to the presence of other components of inward current, about which I will talk later in this lecture.

In fact, the activation and inactivation of the calcium current are both quite rapid when these complications are removed by studying the kinetics of the current in isolated cardiac cells. Figure 2 shows typical results obtained by Lee and Tsien (1982) . The peak inward current occurs after only 3 msec and inactivation is nearly complete after 100 msec. This means therefore that the great majority of calcium entry via these channels occurs during the first 50 mesc of the action potential.

This result leaves two questions to be answered: what carries the much slower inward current that occurs during the plateau, and is
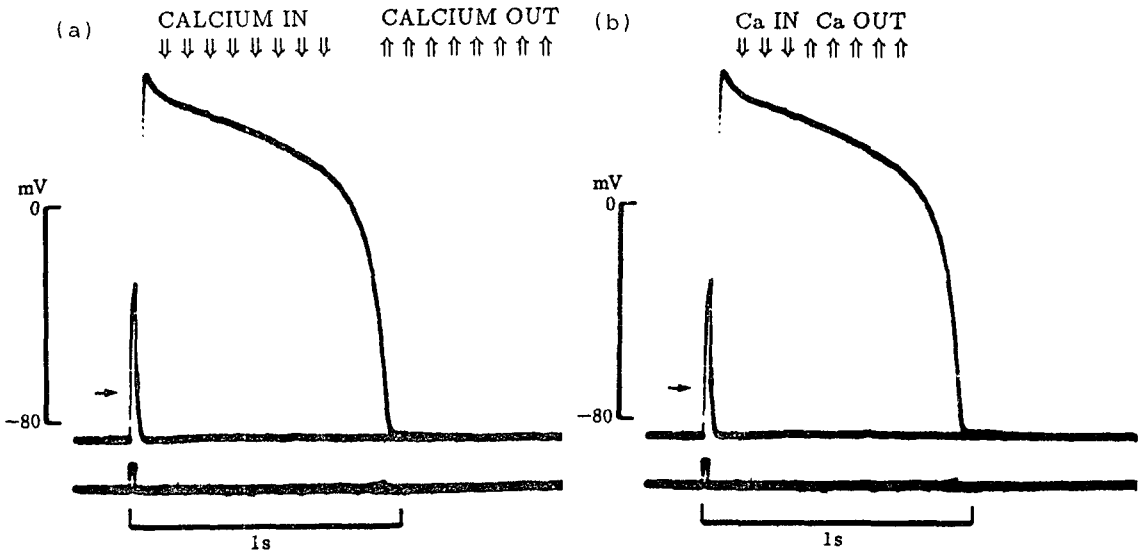
Fig. 1. Two possible modes of calcium extrusion.

    (a) Classical idea: calcium enters during systole and leaves during diastole. This requires very long-lasting calcium current to keep calcium flowing during the whole action potential. Calcium is then pumped out (perhaps via Ca pump) during diastole.

    (b) New idea: calcium current is rapid so that calcium enters during early part of action potential and is extruded during the remainder of the action potential. Only relatively small calcium movements occur during diastole.
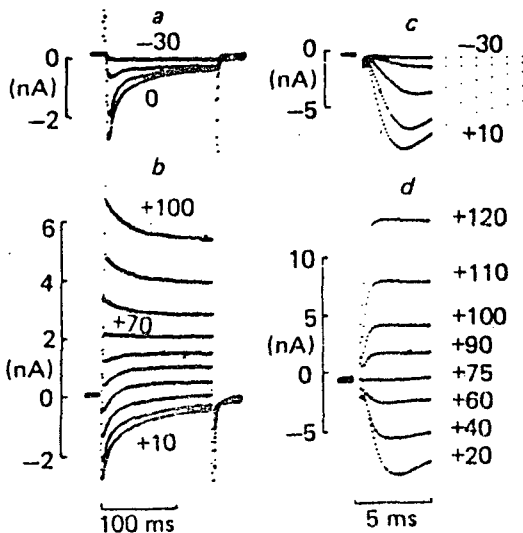
Fig. 2. Calcium currents in isolated guinea-pig ventricular cell (Lee and Tsien, 1982). Peak inward current occurs at 3 msec, and inactivation of nearly complete at 100 msec.

this in any way related to calcium efflux? The hypothesis developed in this lecture is that a slow component of inward current is carried by sodium-calcium exchange and that this is the mechanism that generates calcium efflux.

The sodium-calcium exchange was originally thought to be neutral, exchanging one divalent calcium for two monovalent sodium ions. It is now known that the process is electrogenic and that, in heart, the process carries three sodium ions in exchange for each calcium (see Allen, Noble and Reuter, 1989). There is therefore an excess positive charge should therefore be associated with an inward current.

The most direct measurements of this current were obtained by Kimura, Miyamae and Noma (1987) using isolated single ventricular cells in which the various channel mechanisms had been blocked, leaving only a background current. This current was found to de-

pend on external sodium and internal calcium and to have a characteristic, nearly exponential, current-voltage reation, as shown in Figure 4. The central panel of this figure shows
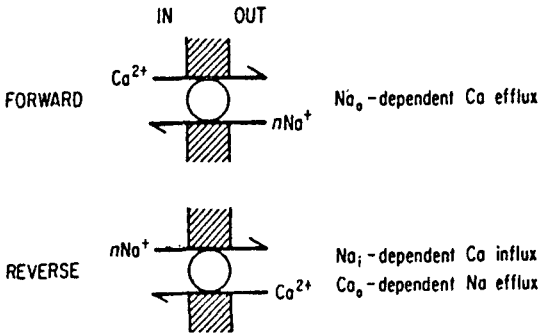

Fig. 3. *Sodium-calcium exchange was first thought to be neutral ($2Na^+ : 1Ca^{2+}$). It is now known to be electrogenic ($3Na^+ : 1Ca^{2+}$), and therefore carries one positive charge in for each calcium pumped out.*

the current mesasured at 140, 70, 35 and 17.5 mM external sodium. The plots in the lower panel show that sodium activates the current with a Km of 70 mM and a Hill plot slope of 3, consistent with three sodium ions binding to the carrier for each cycle of operation. In other experiments, the current was also found to be proportional to internal calcium.

One of the reasons why I was very excited by these results is that these are precisely the properties of the exchange process that DiFrancesco and I (1985) had found were necessary to reconstruct the cardiac action potential. We developed equations from the models proposed by Mullins and found that, with a few simplifications, we obtained equations that predicted exponential voltage dependence for the inward current when the exchange operates in the forward mode.

When we incorporated these equations into our model for the Purkinje fibre action and pacemaker potentials we predicted a slow
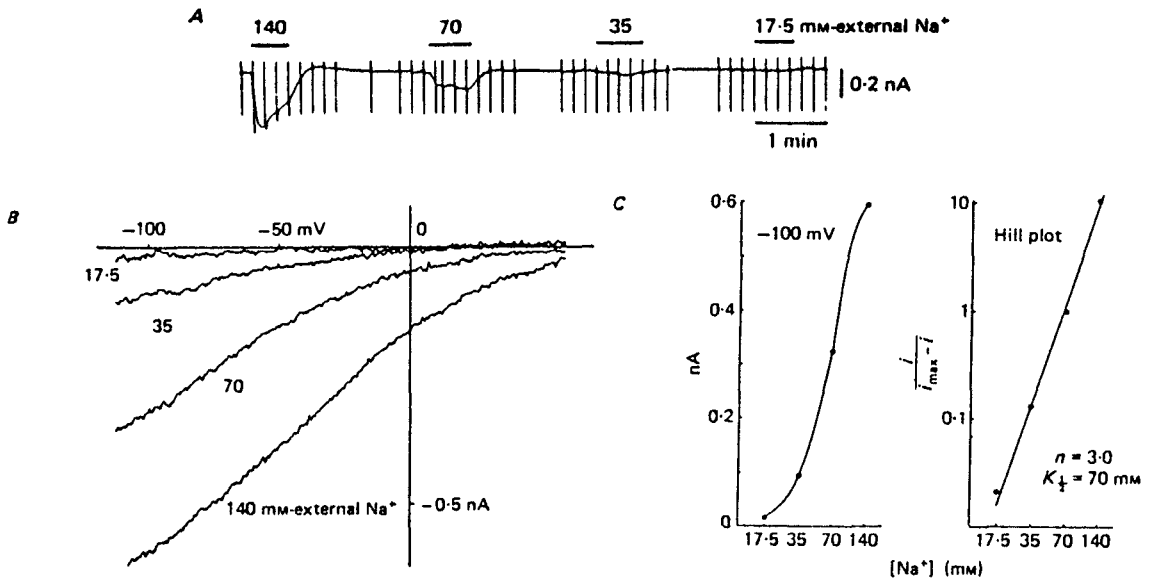

Fig. 4. *Isolation of sodium-calcium exchange current (Kimura, Miyamae and Noma, 1987). When all other currents are blocked with channel blocking drugs and ions, a current remains that depends on $[Na]_o$ and $[Ca]_i$. It shows an exponential dependence on voltage and is proportional to $[Ca]_i$. These happen also to be the characteristics of the equation chosen in 1982 to represent the sodium-calcium exchange process in the DiFrancesco-Noble (1985) model. The current carried is now usually called i_{NaCa}.*
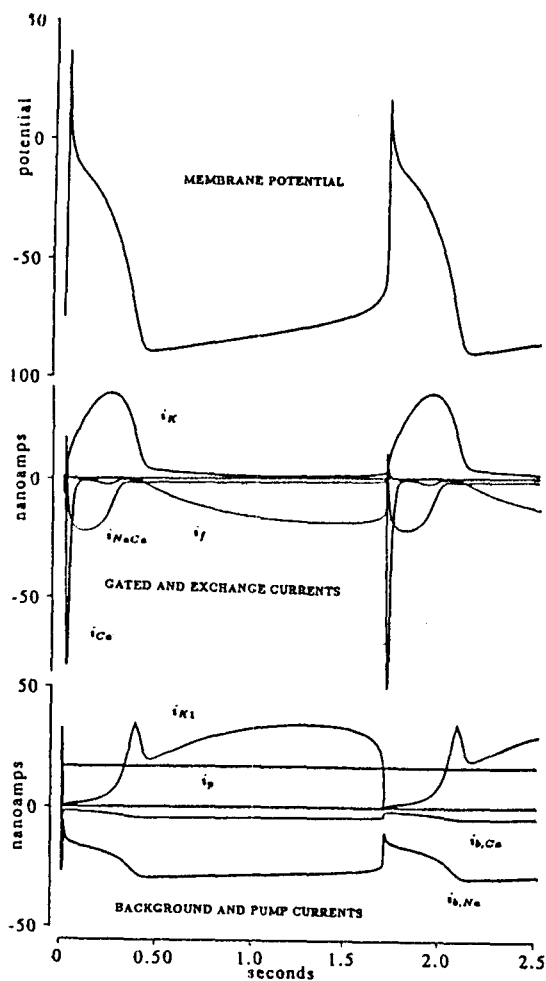
Fig. 5. Original version of the computer model was for the Pukinje fibre (DiFrancesco and Noble, 1985). Note that the model predicts a slow inward current carried by i_{NaCa} after the inactivation of the calcium current, i_{Ca}.
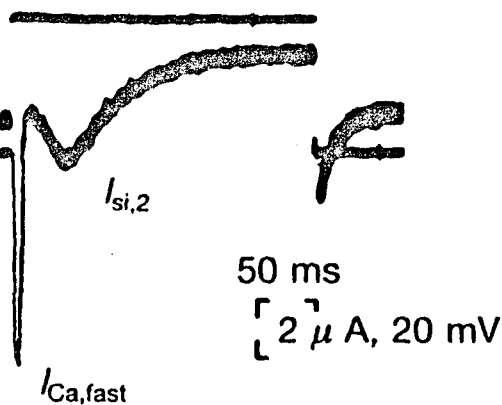

Fig. 6. Voltage clamp experiment in ferret ventricle strip showing slow component (here labelled $i_{si,2}$ but now known to be i_{NaCa}) following the rapid inactivation of the calcium current (here labelled $i_{Ca, fast}$ but now known to correspond to L-type calcium channel, which inactivates rapidly when calcium is large). (Arlock and Noble, 1985).

phase of inward current occurring after the majority of the calcium current inactivation had occurred. This is illustrated in Figure 5, which shows the calculated membrane potential and ionic currents. The calcium current here is labelled i_{Ca}, while the exchange current is labelled i_{NaCa}.

Can such a slow inward current be recorded experimentally? Several examples of such currents have been discussed in Noble (1984)

and in Chapman and Noble (1989). Two examples are shown in Figures 6 and 7. Figure 6 shows a particularly good example obtained in a ferret ventricular strip. In the ferret, the two phases of inward current, attributable to i_{Ca} and to i_{NaCa}, sometimes display two separate current peaks. It is then particularly easy to separate the current components. But even when this in not the case, it is possible to show that a slow phase of inward current is activated by internal calcium. This is illustrated in Figure 7 which shows results obtained in isolated single ferret ventricular cells.
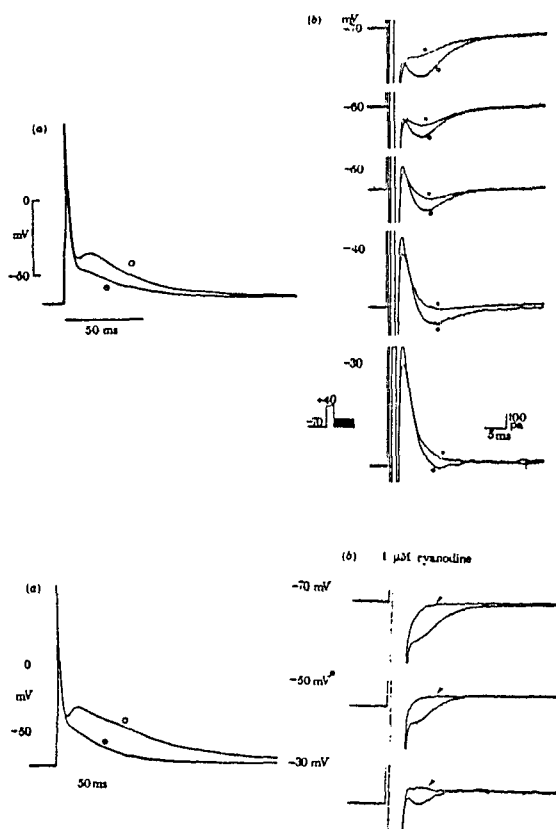
The top row of records shows the membrane current recorded on depolarizing to 0 mV from –40 mV before (A) and after (B) preventing calcium release with ryanodine. Record C shows the two traces superimposed. Ryanodine has clearly removed a slow phase of inward current, though in this case it was not evident as a separate peak. The lower traces show a similar experiment in which the slow component is more pronounced and does
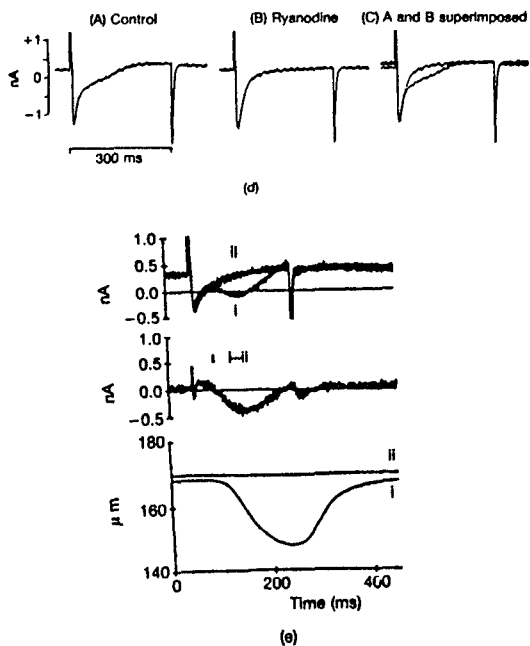
Fig. 7. *Confirmation of Arlock and Noble experiment in isolated single cells from the ferret ventricle (Boyett, Kirby and Orchard, 1989). When contraction is abolished by removing intracellular calcium transient, the slow inward current is also abolished. It is therefore calcium-activated, consistent with it being sodium-calcium exchange.*

Fig. 8. *In rabbit atrial cell,* i_{NaCa} *generates the late low phase of repolarization (Earm, Ho and So, 1990). The current is reduced when external sodium is replaced by lithium, and when calcium release is inhibited with ryanodine, as expected for* i_{NaCa}.

appear as a separate peak. In this case also, removing the calcium transient (and its associated contraction- see lowest records) removes the slow peak. The middle trace shows the difference current, which clearly flows inwards (downward deflection) during the greater part of the depolarization.

Some cardiac cells, such as the atrium and in some species (like the rat) the ventricle, show action potentials with two distinct phases of repolarization. In these cases it has now been shown that the slow phase is dependent on the current carried by sodium-calcium exchange. Figure 8 shows results obtained here in Korea using rabbit atrial cells. The top left records show the action potential recorded in an isolated cell before (○) and after (●) re-

moving sodium-calcium exchange by replacing external sodium with lithium ions. The right hand records show the result of similar substitution on the slow inward membrane current recorded on repolarizing to various membrane potentials after first depolarizing briefly to +40 mV to simulate an action potential. The slow current is clearly greatly reduced in amplitude, particularly at potentials (negative to –40 mV) where the late phase of repolarization occurs.

The lower traces show a similar result obtained by removing the calcium transient with ryanodine. This completely eliminates both
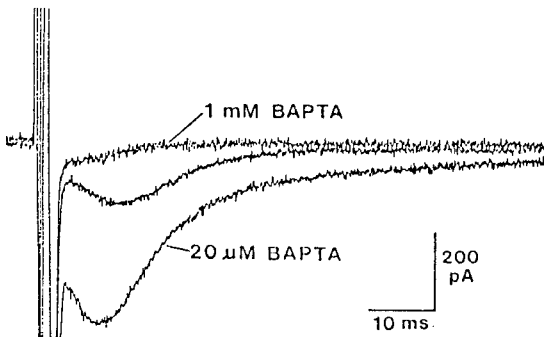
Fig. 9. The slow inward current is also abolished by buffering the iternal calcium with the fast calcium buffer, BAPTA (Earm, Ho and So, 1990).

the late plateau and the slow current recorded at – 30, – 50 and – 70 mV.

Figure 9 shows a particularly elegant experiment in which the slow current was recorded in an isolated atrial cell at different concentrations of the rapid calcium buffer BAPTA. Initially, a very low concentration (20 μM) was used. ImM BAPTA was then infused intracellulary and the current recorded during (middle trace) and after (top trace) the changeover. This concentration almost completely eliminates the calcium-dependent current. This result is important since BAPTA has similar calcium buffering properties to the calcium indicator fura-2 and there is already evidence (see Earm and Noble, 1990; Noble and Powell, 1990) that fura concentrations in this range significantly affect the calcium transient and the relation between calcium current and calcium release.

It is particularly fortunate that the experiments shown in Figures 8 and 9 should have been performed on rabbit atrial cells since the rabbit atrium has already been used to measure fast external calcium transients in order to determine the time course of net membrane calcium flux during the action potential. Moreover, this work has led to the construction of a model of rabbit atrial tissue (Hilgemann and Noble, 1987). Recently, this
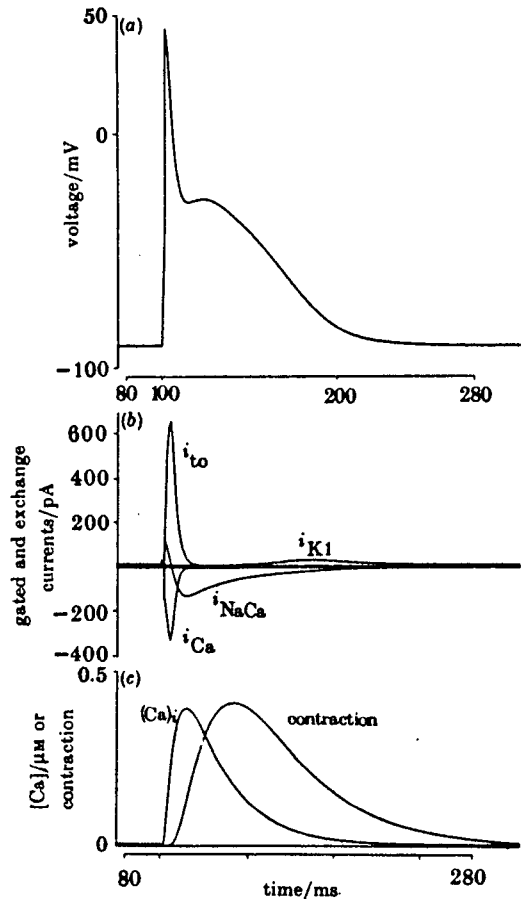
Fig. 10. Computer model reconstruction of rabbit atrial action potential (Earm and Noble, 1990) using a single cell version of a model developed by Hilgemann and Noble (1987). Note that i_{NaCa} is the main inward current throughout the duration of the late low plateau.

model has been adapted to represent a single atrial cell. The result is shown in Figure 10.

The top record shows the computed action potential, the middle traces show some of the computed ionic currents, while the lower traces show the computed internal calcium transient and the contraction. The initial relatively fast repolarization is achieved by two processes: the inactivation of i_{Ca} and the activation of a large transient outward cur-

rent, i_{to}. The low plateau phase is maintained by the sodium-calcium exchange current, i_{NaCa}, with the inward rectifier current i_{K1} contributing to the last phase of repolarization.

These experimental and theoretical results lead to a clear experimental prediction. If the calcium current inactivates as rapidly as shown in Figure 10, and if the remaning inward current is carried by sodium-calcium exchange then the net membrane calcium flux shlould be inward during the first 30~50 msecs and outward during the remainder of the action potential. This is a rather surprising prediction since it means that in action potentials of this shape net calcium efflux occurs very early and lasts much longer than the period of net calcium influx.

Can this prediction be tested? It is impossible to measure calcium membrane fluxes using radioactive calcium with sufficient time resolution to test this prediction. It is necessary therefore to obtain the information more indirectly using very rapid calcium indicators. Measuring internal calcium transients does not give us the required information since the analysis of such transients requires separation of surface membrane from internal membrane fluxes (e.g. across the membranes of the sarcoplasmic reticulum and the mitochondria). The best approach therefore is to measure external calcium since, in this case, there are only two possible ways in which it may change: transmembrane flux or extracellular diffusion. the latter is very slow compared to the speed of transmembrane fluxes.

Figure 11 (top right) shows a result extracellular calcium indicator, tetramethylmurexide. There are three records: the action potential, contraction (motion signal) and the external calcium transient. The preparation here is a multicellular one, so there are narrow intercelluar spaces in which calcium accumulation and depletion can occur. The result is exactly as predicted: calcium depletion (corresponding to net calcium entry into the cells) occurs almost immediately after the action potential upskroke, and continues for about 30
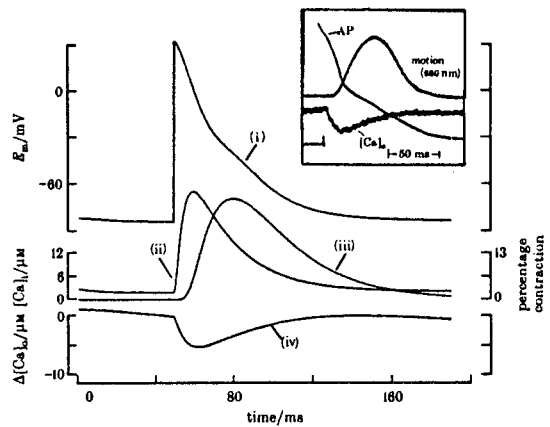
Fig. 11. Test of prediction (Hilgeman and Noble, 1987). The top picture (inset) shows experimental result measuring action potential, contraction and external calcium. Bottom graphs show the computer reconstruction. Notice in both cases that [Ca]$_o$ falls during first 30 msec and then rises again to reach original steady state value at the end of repolarization.
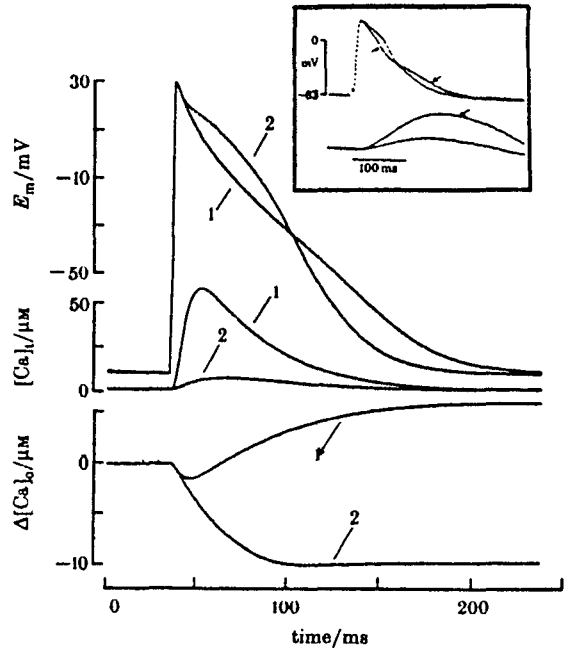
msec. After this time, net calcium reaccumulation occurs, which means that calcium efflux is occurring. This process is complete by the end of repolarization. The main panel of this figure shows the computed result. It can be seen that the computed external concentration (trace iv) closely mimics the experimental result.

There is a second testable prediction: this is that under conditions in which the calcium transient is small or eliminated, calcium efflux should be small and the external calcium signal should then show depletion followed by little or no reaccumulation. A simple physiological manoeuvre to achieve this condition is to use double or multiple stimulation at a frequency high enough to ensure that during the second and subsequent excitations calcium release from the SR is inactivated. Experimental and computed results of this kind are shown in Figure 12. The experimental record (top) shows a large contraction following the
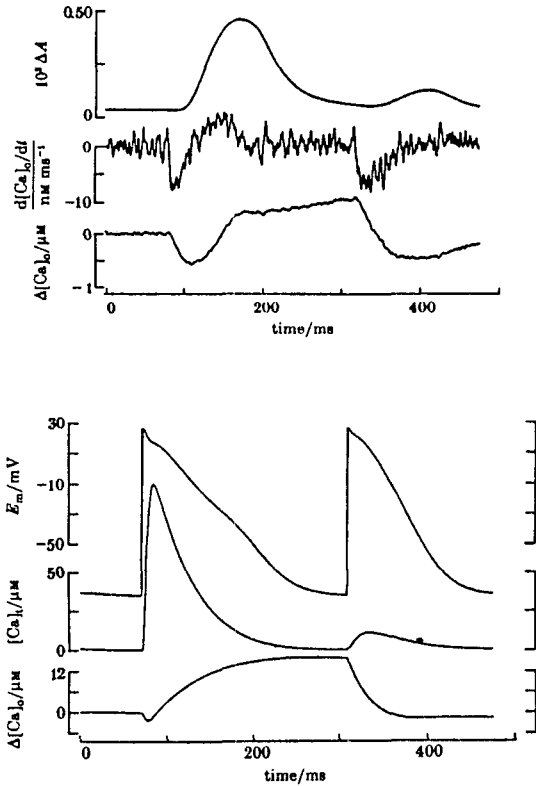
Fig. 12. *If a second pulse is applied while the SR calcium release is inactivated, there is very little contraction. There is then also only a small calcium efflux. (Hilgemann and Noble, 1987).*

Fig. 13. *Here, the first and second action potentials are shown superimposed. The inset (top right) shows experimental result (Hilgemann and Noble, 1987). The variation in the shape of the action potential between the first and second beats is well reproduced by the model.*

first excitation and a very small contraction during the second. As expected the external calcium signal shows depletion followed by reaccumulation during the first excitation, while during the second there is a large depletion with only very little reaccumulation. Clearly, the calcium efflux has been reduced to very small levels during the second beat. The lower traces show a computed result. The initial calcium depletion in this case is almost immediately masked by large reaccumulation corresponding to massive calcium efflux. This arises from the fact that the model was allowed to 'rest' for a long period before being excited. The calcium stores are then heavily charged and a large (rested state) internal cal-

cium transient occurs. During the second beat, the calcium signal is very small. Calcium efflux is then negligible i.e. the external calcium signal stays flat after the initial depletion.

It is interesting to superimpose the action potentials in the first and second beats, since this reveals the extent to which the action potential shape depends on the intracellular calcium transient. This has been done in Figure 13. The inset shows an experimental result. The top traces show the superimposed action potentials while the bottom traces show the contractions. Notice that the first response (indicated by the arrows) initially repolarizes faster. The later phase of repolarization is however slower, so that the two action potentials cross each other at about − 25 mV. The larger panel shows the corresponding comput-

ed records for the action potentials, internal and external calcium transients. The similarity between the computed and experimental action potentials is obvious. The slower late phase of repolarization in the first action potential is, of course, attributable to the inward current generated by sodium-calcium exchange in response to the large internal calcium transient. The faster initial repolarization is attributable to another calcium-dependent mechanism: calcium current inactivation is calcium-dependent and proceeds more rapidly when internal calcium is high.

Figure 14 shows that this analysis also applies to the very similar action potential recorded in rat ventricular cells. The left hand records show that the late plateau is removed when sodium-calcium exchange is reduced by replacing external sodium with lithium ions. The right hand traces show that ryanodine (which blocks calcium release and so abolishes contraction-top traces) also removes the late plateau.

The rabbit atrium and rat ventricle are convenient tissues for this analysis since they display two nearly distinct phases of repolarization and, as we have now shown, they can be attributed respectively to the flow of calcium current and sodium-calcium exchange current. In many species however, including man and guinea-pig. ventricular cells do not display this behaviour. Instead, the repolarization process is initally extremely slow, with a faster phase at the end of the action potential. This generates the typical square plateau already illustrated in Figure 1. In such cases there can be no neat separation into phases primarily dependent on i_{Ca} and i_{NaCa}. Can the contribution of i_{NaCa} nevertheless be determined?

Figure 15 shows how this question can begin to be answered. If action potential is interrupted by applying a voltage clamp at the resting potential, a slow 'tail' current is recorded. This tail current is abolished by buffering internal calcium, e.g. with EGTA or BAPTA. Its amplitude depends on the inotropic state of the cell. A large contraction is accompanied by a large tail current. Thus, if action potentials are continuously interrupted (○) then the sarcoplasmic reticulum stores do not have an opportunity to be recharged so the calcium release becomes weak and a relatively small tail current is obtained. Whereas, if we interrupt only alternate action potentials (●) leaving full action potential in between to recharge the SR with calcium, then the contraction is strong and the tail current is much larger (●).

The current obtained in this way is clearly calcium-dependent. In fact its time course closely follows that of the internal calcium transient. This is shown by the experiments illustrated in Figure 16, where the voltage clamp interruptions have been applied at different times during repolarization. If the tail
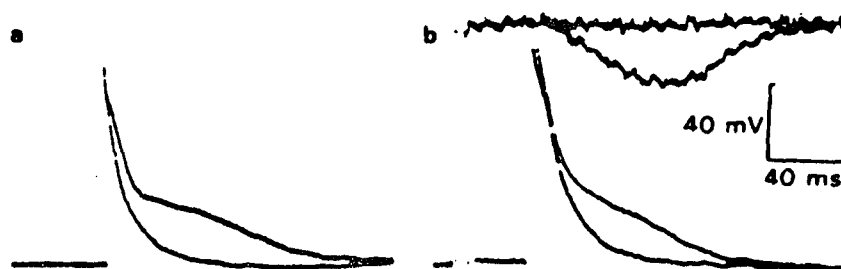
Fig. 14. *Similar results also obtained in rat ventricular cell (Mitchell, Powell, Terrar and Twist, 1985). Here also the late low plateau is reduced by replacing external sodium with lithium or by inhibiting calcium release with ryanodine.*
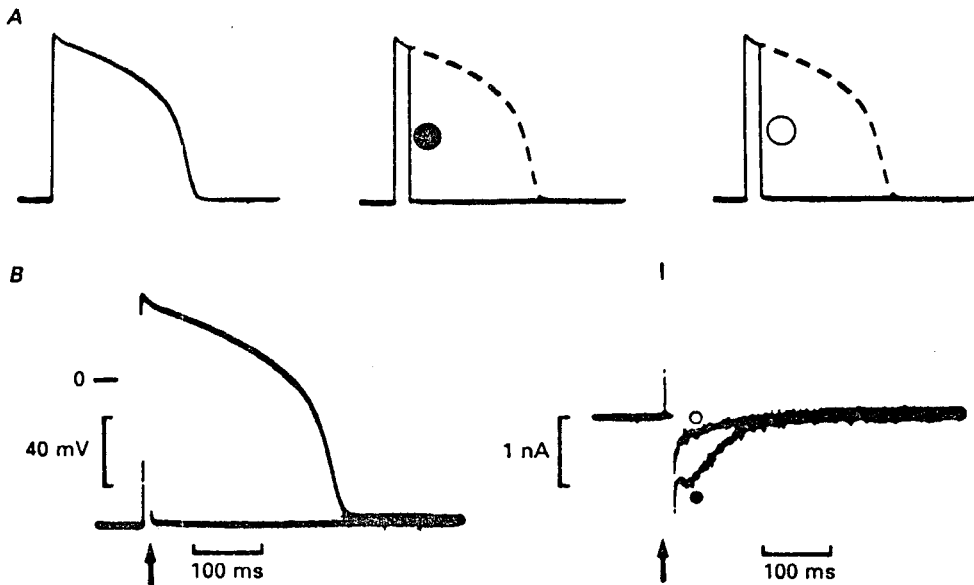
Fig. 15. *Exchange current 'tails' recorded in guinea-pig ventricular cells (Egan, Noble, Powell, Spindler and Twist, 1989). The method used here is to interrupt the guinea-pig action potential at various times with voltage clamp steps to the resting toptential (– 80 mV). A slow inward current 'tail' then develops. This current is abolished when the calcium transient is abolished. It therefore represents i_{NaCa} not i_{Ca}.*
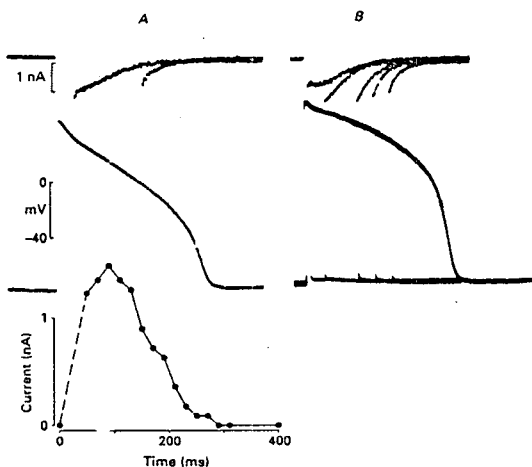

Fig. 16. *By interrupting at different times during the action potential the time course of activation of i_{NaCa} can be determined (Egan et al, 1989). It is very similar indeed to the time course of [Ca]$_i$ measured with calcium indicators.*

current amplitudes are plotted against time, the result is a curve (lowest part of figure) that rises within about 50 msec and then decays to nearly zero by the end of repolarization. This is very similar to the time course of [Ca]$_i$ measured with internal calcum indicators such as fura-2.

With this information it is possible to make an approximate reconstruction of the time course of the exchange current during the action potenial itself. There are three assumptions involved in doing this:

1. That there is a direct relationship between [Ca]$_i$ and the exchange current. This is the case, and the data obtained by Kimura, Miyamae and Noma (1987) show that about 1 nA of current is generated by each μM of internal calcium. With this assumption it is possible to convert the exchange current tail amplitudes into the underlying variation in internal calcium. This has been done in Figure 17, which shows that the peak internal calcium is

A

B
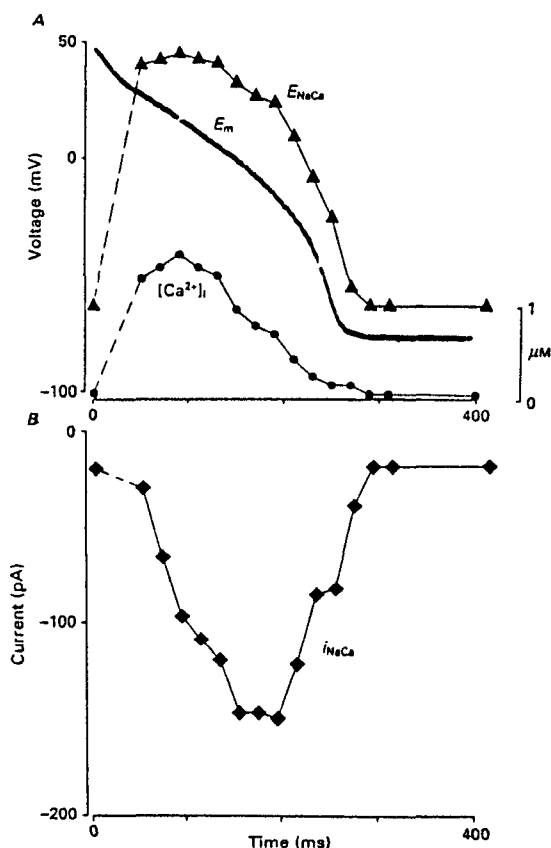
Fig. 17. *Here, the time course of i_{NaCa} during the plateau and repolarization has been calculated using the experimental results shown in the previous figures (Egan et al 1989). There is net inward current, and therefore net calcium efflux, during the entire time course of repolarization except for a brief period at the beginning of the action potential.*

around 1.25 μM, close to that recorded experimentally.

2. If an assumption is now made for the value of [Na]ᵢ it is possible to calculate the reversal potential for the exchange current. This has also been done in Figure 17, where the assumed value was 7 mM. Note that, apart from a brief period at the beginning of the action potential, the reversal potential is positive to the membrane potential throughout

repolarization, which means that the exchange current will flow inwards to extrude calcium throughout this period of time.

3. That the effect of voltage changes on the exchange current is immediate and is well represented by the current-voltage relations already shown in Figure 4. This assumption leads to the calculated exchange current shown as the bottom graph in Figure 17. There is a brief period (indicated by the interrupted line) during which the calculation is very uncertain. The exchange current may even have been outward during this period, but the data is not accurate enough to be sure. The current then rises to a peak inward value of about 150 pA half-way through the action potential, before declining to nearly zero by the end of repolarization.

We may conclude therefore that, although a separate phase of repolarization strongly dependent on the exchange current could nevertheless be sufficient to extrude calcium during the action potenial.

Finally, in this lecture, I want to ask whether the exchange current may play a role in arrhythmias. This possibility has existed for some time, since Lederer and Tsien (1976) first showed that ectopic beats during glycoside toxicity are attributable to an oscillatory transient inward current. This current has been shown to be activated by internal calcium oscillations that occur in conditions of calcium overload. The main controversy has concerned whether the calcium-activated current involved is the sodium-calcium exchange or a calcium-activated cation channel. Both mechanisms can be activated depending on the level of internal calcium involved.

It is therefore important to determine what happens in ventricular cells when the transient inward current is activated. Figure 18 shows an experiment designed to answer this question. The current was induced by a short depolarizing pulse to +35 mV from a holding potential of –20 mV in a calcium-overloaded cell. After repolarization there is an inward tail current followed by inward current oscilla-
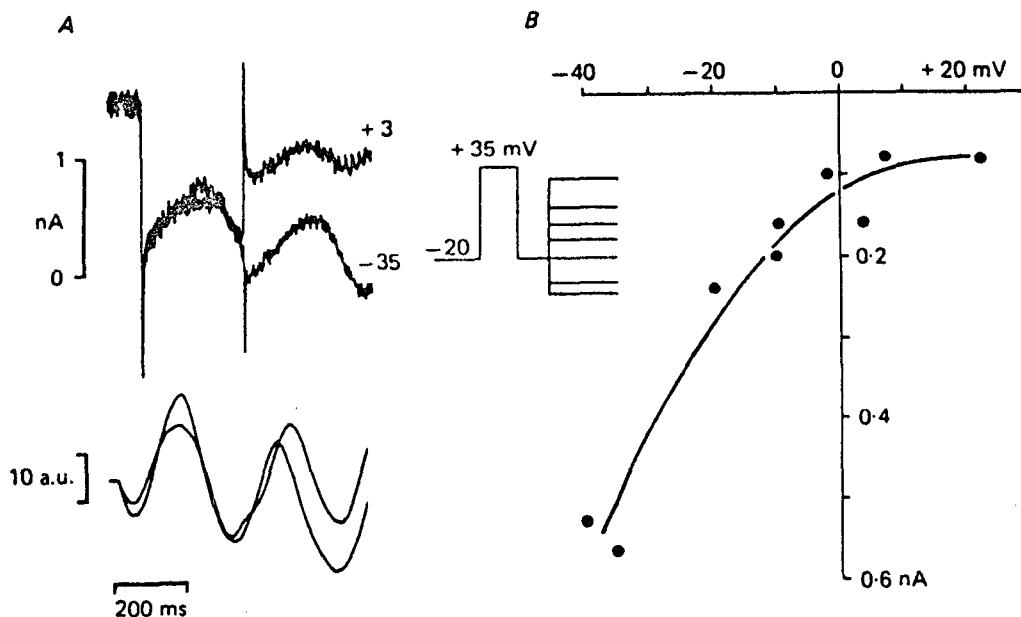
Fig. 18. *Sodium-calcium exchange also carries the arrhythmogenic current, i_{TI} (Fedida, Noble, Rankin and Spindler, 1988). In calcium overload conditions (e.g. during blockade of the sodium-potassium pump) calcium oscillations occur. These oscillations generate ectopic beats by activating oscillartoy inward current carried by sodium-calcium exchange. The current-voltage relation of the transient inward current resembles that determined for the Na-Ca exchange by Kimura, Miyamae and Noma (1987).*

tions and accompanying contractions. At the peak of the inward current, the voltage was stepped to various levels and the size of the inward current oscillation was measured. The relastionship between this current oscillation amplitude and the membrane potential is plotted on the right. It can be seen that the current-voltage relationship resembles that for the exchange currrent (see Figure 4). In particular there is no reversal potential, as would have been expected if a cation channel were involved. The conclusion is that in these conditions the great majority of the transient inward current is attributable to sodium-calcium exchange.

Why though does internal calcium oscillate when calcium-overload occurs? After all, normal release of calcium from the SR in not oscillatory; there is a single release in response to the calcium flowing in via the calcium cur-

rent during normal heart beats. One possibility is that there may be some degree of positive feedback between cytosol calcium levels and calcium release, perhaps insufficient to produce regenerative or oscillatory behaviour at low levels of free calcium, but sufficient to do so at high levels. There is evidence that the relation between calcium current and calcium release is not linear (for discussion of this question see Earm and Noble, 1990), and Fabiato showed that when sufficient calcium is applied to the SR in skinned cardiac cells, oscillatory waves of release can be induced.

All the essential features of calcium-induced calcium release are already incorporated into the models I have used previously in the lecture. It is time therefore to put them to a very severe test. Will they show oscillation when the model cells are subjected to calcium overload? This question is answered in Figure 19,
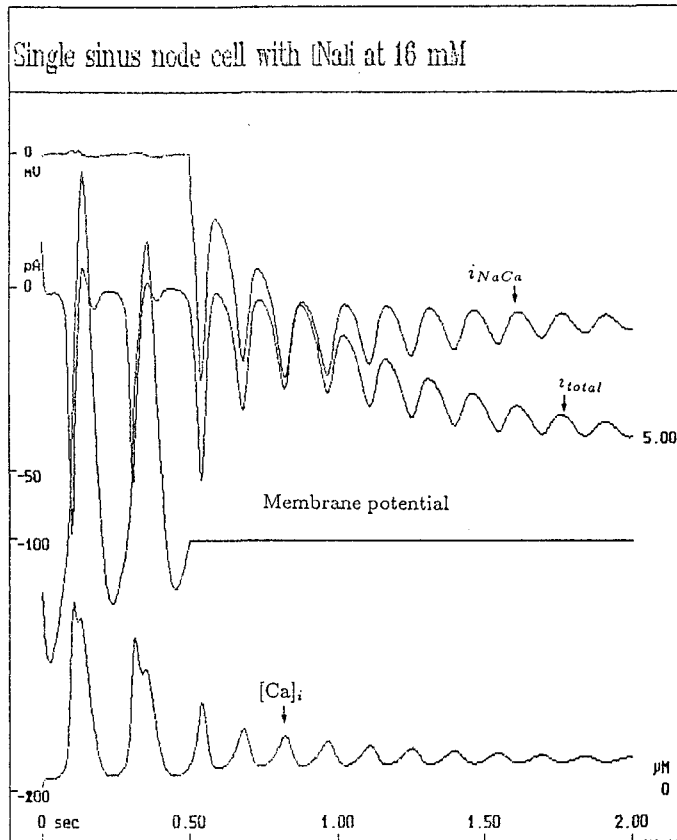
Fig. 19. *In this calculation the single sinus node cell model was run with internal sodium allowed to rise to 16 mM from its normal value of 7 mM. For the first 500 msec the model is allowed to generate action potentials. It does this spontanously, but the responses are not normal ones. They are generated by the calcium transient itself. Notice that the calcium transient rises before the action potential usptroke. Moreover, when the voltage is clamped at 0.5 secs the calcium oscillations continue.*

which shows the behaviour of the single sinus node cell mode when internal calcium is raised by increasing internal sodium from its normal level of 7 mM to 16 mM, i.e. the level of sodium increase induced, for example, by blockade of the sodium pump. It is clear that this induces calcium oscillation since, when the model is clamped after the first two beats, the calcium oscillations continue. They are not therefore generated by the calcium current. They are generated by instability in the internal sequestration and release mechanisms once free calcium is allowed to rise sufficient-

ly. Similar oscillations can also be obtained in the Purkinje and atrial cell models described earlier in this lecture.

Finally, does the current induced in this way become sufficient to induce ectopic beats? The last figure 20 shows an calculation done on the Purkinje model with internal sodium set to 17 mM. Each oscillation of internal calcium generates a transient inward current sufficient to induce a substantial depolarization. Each third response is sufficient in this case to induce an ectopic beat. At 15mM internal sodium the oscillations induced ec-
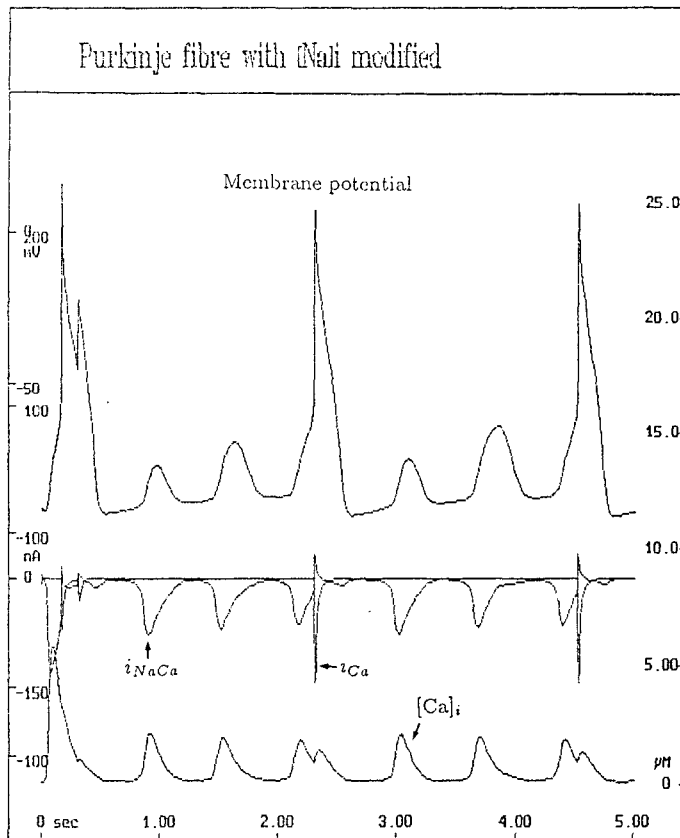
Fig. 20. *Initiation of ectopic beats in Purkinje fibre model. Internal sodium was raised to 17 mM. This immediately generates a release of internal calcium which activates sufficient sodium-calcium exchange current to depolarize the membrane to the threshold for initiating an ectopic beat. A stimulus was programmed to arrive during the ectopic action potential (to respresent an arriving sinus beat) but fails to initiate a second action potential since it arrives during the refractory period. The subsequent calcium oscillations generate oscillatory sodium-calcium exchange current which sometimes produces abortive transient depolarizations and sometimes (every third beat) initiates an ectopic action potential.*

topic beats every time. Clearly then the model does reproduce ectopic beats arising from calcium overload.

CONCLUSIONS,

The conclusions of this lecture are very clear:

1. Calcium current is activated and inactivated relatively quickly.

2. This calcium current induces calcium release by the sarcoplasmic reticulum.

3. The rise in [Ca]$_i$ activates sodium-calcium exchange to pump calcium out of the cell.

4. This generates a slow phase of inward current.

5. In conditions of calcium-overload (pump block, ischaemia etc) the calcium release becomes oscillatory.

6. This generates the transient inward current and ectopic beats.

ACKNOWLEDGEMENTS

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