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RELATIONSHIP BETWEEN THE TRANSIENT INWARD CURRENT AND SLOW INWARD CURRENTS IN THE SINO-ATRIAL NODE OF THE RABBIT

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SUMMARY

1. In low K^+ (0·3 mM) solutions rabbit sinus node preparations show the oscillatory transient inward current, i_{TI}, already recorded in these conditions in Purkinje and ventricular preparations.

2. The time course of i_{TI} closely resembles that of the slow component of the slow inward current (i_{si}) previously reported by us (Brown, Kimura, Noble, Noble & Taupignon, 1984*a*) in rabbit sinus node, when recorded near its threshold (−40 mV). When the duration of voltage-clamp steps is varied there is a strong correlation between the 'envelope' of i_{si} amplitudes on depolarization and the time course of i_{TI} on hyperpolarization.

3. Although oscillations of i_{TI} become smaller near 0 mV, there is no potential at which the current records are completely flat, suggesting that there is no simple reversal potential.

4. 75% substitution of Na^+ by Li^+ greatly reduces both i_{TI} and slow i_{si} in about the same proportion.

5. Reducing the activity of the Na–K exchange pump by the amount expected in 0·3 mM-K^+ solutions is sufficient to induce oscillatory i_{TI} in a computer model of the sino-atrial node (Noble & Noble, 1984). The model reproduces the current as variations in the Na–Ca exchange current dependent on intracellular Ca^{2+} concentration ($[Ca]_i$).

6. The model was also used to test the alternative hypothesis that the slow inward currents might be generated by $[Ca]_i$-activated non-specific cation channels. It is shown that this would distort the shape of the repolarization phase of the action potential.

7. It is concluded that the experiments and computations are consistent with the hypothesis that a large fraction of i_{TI} and the slow component of i_{si} could both be generated by Na–Ca exchange and that only a relatively small fraction might be generated by non-specific channels.

INTRODUCTION

In conditions of Ca^{2+} overload a damped oscillatory current, the transient inward current (i_{TI}), appears in cardiac cells. It was first described by Lederer & Tsien (1976) in the sheep Purkinje fibre and has also been reported in mammalian ventricle by Karagueuzian & Katzung (1982).

The genesis of transient inward currents is well known. Reduced activity of the Na–K exchange pump (as is caused, for example, by low external K^+ concentrations) leads to a rise of intracellular Na^+ concentration and, since the Na^+ gradient is reduced, to decreased Na–Ca exchange and hence a rise in intracellular Ca^{2+}.

Kass, Lederer, Tsien & Weingart (1978) suggested two possible Ca-activated mechanisms for the transient inward current: (1) activation of Na–Ca exchange (which would result in a net inward current if the stoicheiometry favours Na^+) or (2) opening of non-specific cation channels of the type recently demonstrated in patch-clamp studies by Colqhoun, Neher, Reuter & Stevens (1981).

The oscillatory nature of the current may be attributable to Ca-induced release of Ca^{2+} from internal stores, as has been shown by Fabiato & Fabiato (1975) in skinned muscle cells (see also review by Fabiato, 1983). Oscillatory release has also been detected in recent studies using aequorin (Allen, Eisner & Orchard, 1984).

The transient inward current is usually regarded as an additional current occurring during Na-pump blockade. The question, though, arises whether the variations in Ca^{2+} concentration during normal rhythmic activity might also activate inward current by the same process(es), the only differences being that the normal Ca^{2+} transient is not oscillatory and that the current it may generate could be small enough to be masked by the activation of the much larger Na^+ or Ca^{2+} channel currents.

We have recently presented evidence for the hypothesis that the slow inward current, i_{si}, consists of at least two components: a first, faster component (which we call $i_{Ca,f}$) and a second slower component (which we call $i_{si,2}$) (Brown, Kimura, Noble, Noble & Taupignon, 1984*a*, *b*; Noble, 1984). $i_{Ca,f}$ comprises Ca^{2+} entry through gated channels, while the nature of $i_{si,2}$ is more controversial. Using a computer model of sino-atrial node electrical activity (Noble & Noble, 1984) developed from the extensive modelling work of DiFrancesco & Noble (1982, 1985), we have been able to reproduce the experimental records of $i_{si,2}$ very successfully on the assumption that the current is activated by the intracellular Ca^{2+} transient and that it is carried by the Na–Ca exchange process.

If this model is correct then there should be a correlation between the properties of the slower phases of i_{si} and the transient inward current. In the present paper we report the results of experiments designed to investigate this possibility.

METHODS

Small (250 μm) preparations of rabbit sino-atrial node were voltage clamped using the two-micro-electrode method. Details of this method have been described previously (Noma & Irisawa, 1976; Brown & DiFranceso, 1980; Kimura, 1982).

Solutions

Normal Tyrode solution. NaCl, 140 mM; KCl 3 mM; $MgCl_2$, 1 mM; $CaCl_2$, 1·8 mM. Buffered with Tris HCl(Sigma) to pH 7·4. Saturated with 100% oxygen.

'*Low K^+*' *Tyrode.* Same as above but KCl, 0·3 mM.

Low K^+ low Na^+ Tyrode. KCl, 0·3 mM; 75% of the NaCl replaced by equimolar LiCl.

Drugs

TEA (Sigma). Added at 30 mM. TTX (Sigma). Added at 2×10^{-6} g/ml.

Computer simulations

These were run on a PDP computer using the computer program Oxsoft Heart version 1.0. This incorporates the sino-atrial node model developed in this laboratory (Noble & Noble, 1984).

RESULTS

A. Experimental results

Exposure to low K^+ (0·3 mM, i.e. one-tenth the concentration in normal Tyrode solution) results after about 5 min perfusion in the development of transient inward currents in the rabbit sino-atrial node similar to those seen in conditions of Na–K-pump

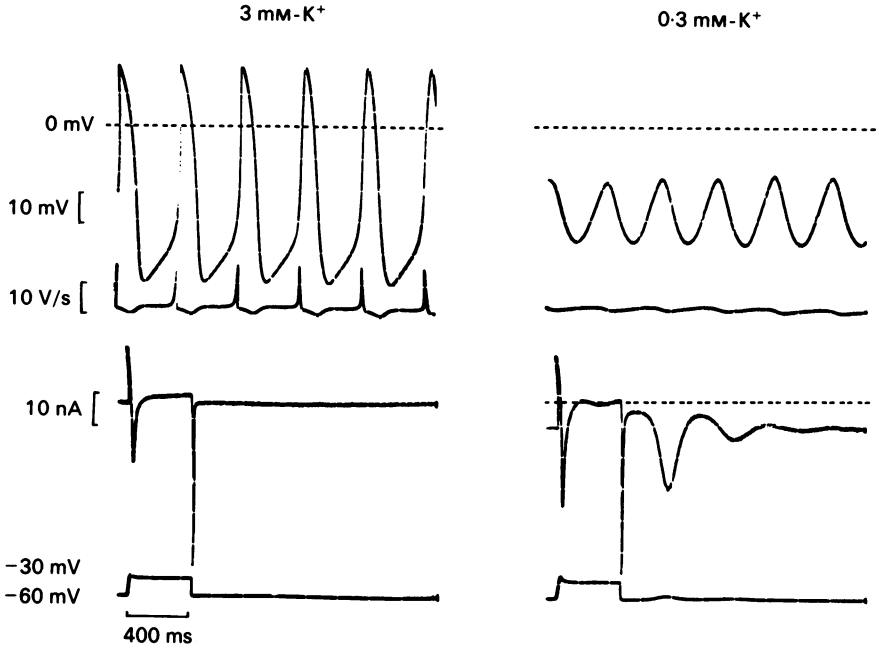

Fig. 1. Rabbit sino-atrial node preparation. Unclamped electrical activity and rate of change of voltage (top two traces); current traces and voltage-clamp pulses (lower two traces) in normal (3 mM–K^+) Tyrode solution (left) and after 7 min in low K^+ (0·3 mM–K^+) solution (right). Note the oscillatory transient inward currents during and, more noticeably, after the voltage-clamp pulses in low K^+. The dotted line in this current trace (and in subsequent Figures) represents zero current level; the inward current movement is to be expected as the preparation tends to depolarize in low K^+ solution. TTX (2×10^{-6} g/ml) and TEA (30 mM) in this and all other experiments.

block and consequent intracellular Ca^{2+} concentration ($[Ca]_i$) increase in other cardiac tissues (Eisner & Lederer, 1979). Fig. 1 shows a sino-atrial node preparation in normal (3 mM–K^+) Tyrode solution and after 7 min perfusion with low K^+ (0·3 mM) Tyrode solution. Unclamped activity is shown above; voltage-clamp pulses, in each case from -60 to -30 mV are shown below. In low K^+, the normal pace-maker

activity (pace-maker depolarizations leading to action potentials) is replaced by small amplitude oscillations. Under voltage clamp, oscillatory transient inward currents can be seen, both during and, more strikingly, after the voltage-clamp depolarizations.

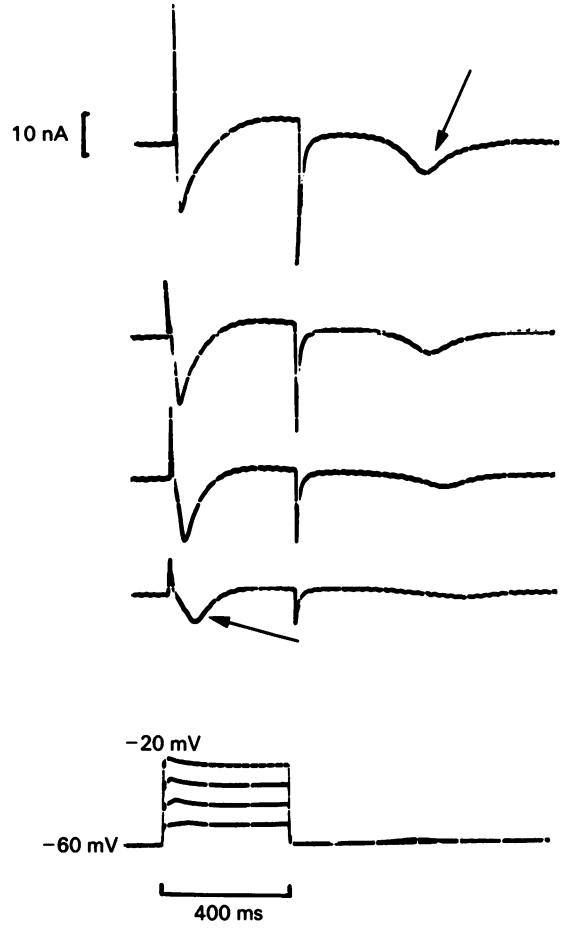

Fig. 2. Voltage-clamp depolarizations (below) given to a rabbit sino-atrial node preparation from a holding potential of −60 mV, and corresponding current traces (spread out for clarity) above. The arrows point to the slow inward current during the smallest depolarizing pulse (to −50 mV) and to the transient inward current after the largest depolarization (to −20 mV). Note that they have very similar time courses. Note also how the time course of i_{si} becomes faster as the depolarizing pulses become larger.

K^+-depleted solutions usually first induced a single transient inward current oscillation after each depolarizing pulse. As the preparation remains longer in the low K^+ solution, multiple oscillations appear after a pulse and single or multiple oscillations can also be recorded during the pulse. The oscillations show a symmetrical time course and they last for about 200 ms at −60 mV (see, for example, Fig. 1).

The shape and time course of the transient inward current recorded in sino-atrial node are often very reminiscent of those of the slow inward current recorded at

potentials close to the activation threshold of this current. This is illustrated in Fig. 2. Once again the Na–K pump was blocked by exposure to low K^+. When the records shown in Fig. 2 were taken, the preparation had been in the low K^+ for only 6 min, which is probably the reason why transient inward currents were evident only after,

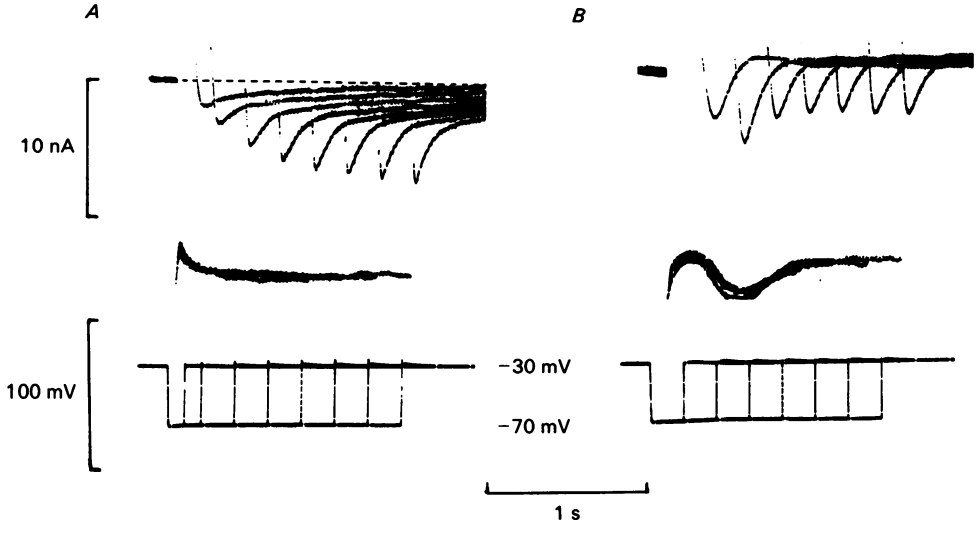

Fig. 3. Currents (above) during and after the hyperpolarizing clamp pulses shown below given to a sino-atrial node preparation from a holding potential of −30 mV. Note that in these records no current trace is seen during the 'on' or 'off' of each pulse. Traces in *A* are in normal Tyrode solution, those in *B* are after 15 min in low (0·3 mM) K^+ solution. On return to the holding potential after each hyperpolarizing pulse slow inward current (i_{si}) is activated and in normal Tyrode (*A*) the 'envelope' of the magnitudes of these slow inward currents increases steadily with the duration of the preceding hyperpolarization. In low K^+ solution (*B*), the i_{si} envelope follows the same oscillatory outline as that of the transient inward current which now develops during the hyperpolarizing pulses.

not during, the voltage-clamp depolarizations. The holding potential was again −60 mV. The slow inward current activated during the smallest (10 mV) depolarization does have a very slow time course. On return to the holding potential after this pulse there is hardly any current oscillation but, as the magnitude of the depolarizations increases, two changes can be seen: the i_{si} activated during each pulse becomes progressively larger and faster and the transient inward current oscillations recorded following each depolarization become more noticeable. The transient inward current recorded after the largest depolarization shown (to −20 mV) bears a striking resemblance to the current recorded during the smallest depolarization (both are arrowed).

The apparent change in the kinetics of i_{si} with increasing magnitude of depolarization, seen in Fig. 2, is often observed in the sino-atrial node. Our interpretation (see Brown *et al.* 1984*a*) is that very small quantities of Ca^{2+} entering the cell either through the 'gated' membrane channel or via the Na–Ca exchange trigger the release of intracellular Ca^{2+}. This in turn produces activation of Na–Ca exchange and, near

threshold, this latter component comprises the major part of the slow inward current. At more depolarized potentials, much larger quantities of Ca^{2+} initially pass into the cells through the gated pathway activating the exchange current more rapidly. The two components can sometimes be recorded as separate entities near threshold but always fuse as the first component becomes larger at more depolarized potentials (see Brown *et al.* 1984*a*).

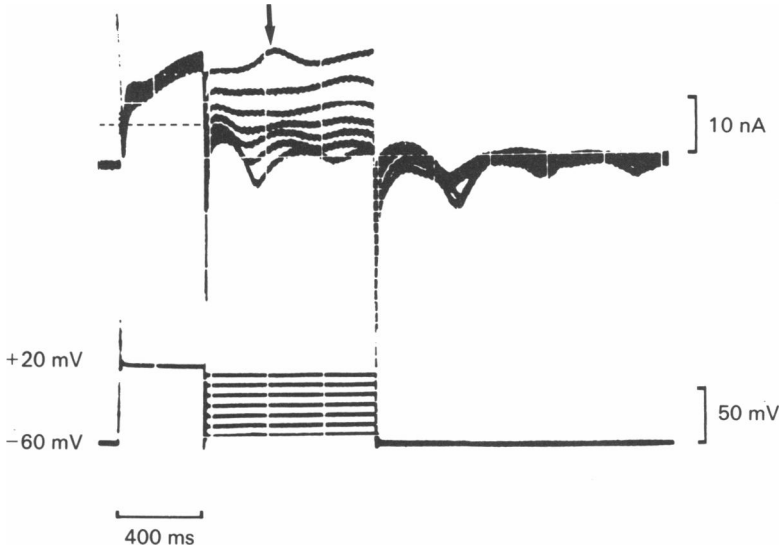

Fig. 4. Sino-atrial node preparation in low K^+ solution for 5 min. A first pulse of 400 ms from the holding potential of −60 mV to +20 mV was immediately followed by a second pulse to potentials between −50 and +10 mV (10 mV steps). The transient inward current oscillations during the second pulse become progressively smaller as the potential level of this pulse is made more positive and the phase of the oscillation shifts. The current trace becomes flatter at 0 mV but there is no potential at which it is completely flat and thus there is no simple 'reversal potential'. This resembles the situation observed in single ventricular cells (see Fedida *et al.* 1985).

Fig. 3 shows more evidence of the similarity between i_{si} and i_{TI}. From a holding potential of −30 mV, a sino-atrial node preparation was given voltage-clamp hyperpolarizations to −70 mV for progressively increasing lengths of time, from 200 ms to 1·4 s. On the left, in control conditions (3 mM–K^+ Tyrode), the magnitude of i_{si} activated on return to −30 mV increases progressively as the duration of the preceding hyperpolarizing pulse is lengthened. Following the peak of the current, a small inward current displacement lasting for several hundred milliseconds can be seen: it becomes larger as the duration of the hyperpolarization is increased. The current during the hyperpolarizations shows no oscillations. On the right, the same procedure was repeated after 15 min perfusion with low (0·3 mM) K^+ Tyrode. The 'envelope' of i_{si} magnitudes instead of increasing steadily, now shows an oscillatory outline which follows that of the transient inward current activated during the hyperpolarizations. The residual component of inward current has now disappeared.

The finding that both i_{si} and i_{TI} follow the same time course in this way supports the hypothesis that they stem from the same underlying mechanism.

If the mechanism of the transient inward current is activation of non-specific membrane channels rather than of Na–Ca exchange, then a clear current reversal should occur at positive potentials. Examination of the records in Fig. 4 shows,

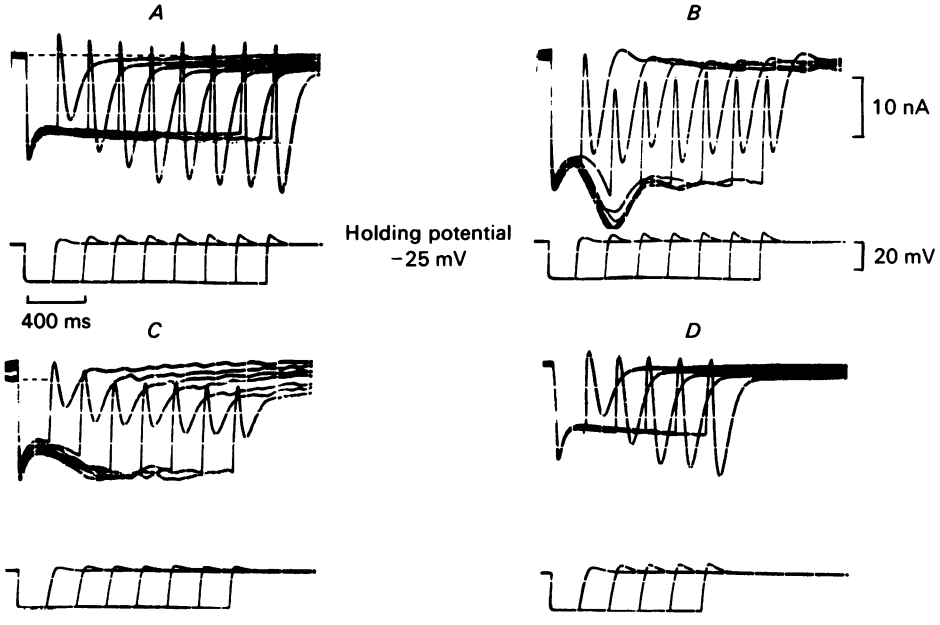

Fig. 5. The same protocol was used as in the experiment shown in Fig. 3, though the holding potential is now −25 mV (rather than −30 mV) and the clamp hyperpolarizations are of only 30 mV (i.e. to −55 mV). In *A* (normal Tyrode), as before, the 'envelope' of the magnitudes of the i_{si} produced after each pulse increases smoothly. In *B* (after 6–7 min in low K^+), this envelope again follows the oscillatory outline of the transient inward currents induced by low K^+. In *C*, 75% of the Na^+ has been replaced by Li^+, with K^+ remaining low. The record was taken after 6 min in this solution: the amplitudes of both the i_{si} and the transient inward current oscillations are much diminished. The marked outward movement of the holding current is as expected in low Na^+ as the preparation hyperpolarizes. *D* shows the same preparation after 8 min back in normal (3 mM–K^+) perfusion fluid.

however, that a clear reversal is not seen. The holding potential was again −60 mV and the membrane was depolarized to −20 mV for 400 ms, then repolarized to potentials varying between +10 mV and −50 mV for a further 600 ms before being returned to the holding potential. Once again, transient inward currents were produced by exposure to low K^+ solution for 5 min. If the second pulse had been terminated at the time marked by the arrow (after 300 ms) it could indeed have been argued that there was a current reversal at 0 mV though such a reversal would have been accompanied by a definite phase shift: the peak of the current occurs after 160 ms at −50 mV while it occurs after 240 ms at +10 mV. Allowing the second

depolarization to continue until 600 ms shows that a clear current reversal is not present at all: the current trace at 0 mV, which is flat during the first 300 ms thereafter shows an oscillation. Moreover, the amplitude of this oscillation, while smaller than at more negative potentials, is still substantial (2·2 nA compared to amplitudes of 2·5 nA at −20 mV and 55 nA at −40 mV).

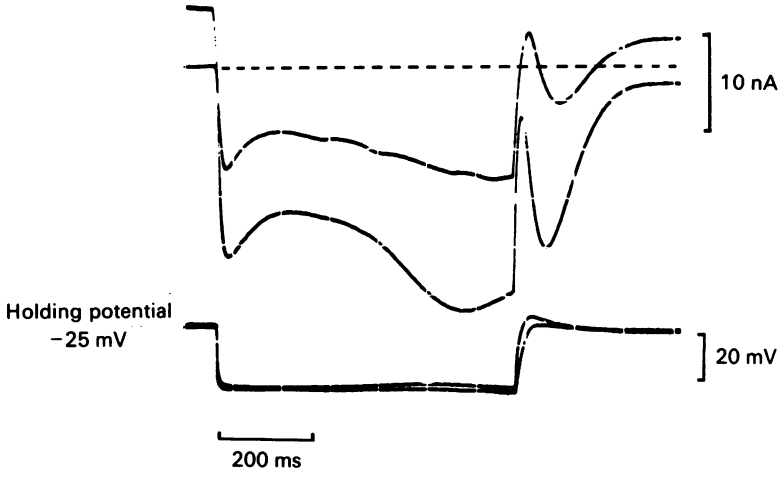

Fig. 6. Same experiment as in Fig. 5. Two hyperpolarizing clamp pulses of 600 ms duration and the corresponding current traces have been recorded on a faster time base and are shown superimposed. One (below) is in low K^+ (as in Fig. 5*B*) and the other in low K^+–low Na^+ (as in Fig. 5*C*). Note diminution of the magnitudes of both i_{si} and i_{TI} in low Na^+.

Li ions are known to pass through Ca-activated non-specific channels (Yellen, 1982), but will not substitute for Na^+ in the Na–Ca exchange process. Replacing 75% of the Na^+ in the perfusing tyrode solution by Li^+ has striking effects on both i_{si} and i_{TI}. Fig. 5 shows 'envelopes' of the magnitudes of i_{si} after voltage-clamp hyperpolarizations of increasing durations (in this case to −55 mV from a holding potential of −25 mV). In Fig. 5*A*, the preparation was in normal Tyrode solution. Fig. 5*B* shows the development of transient inward currents after 6–7 min in low K^+, with the corresponding oscillatory 'envelope' of i_{si} magnitudes. The perfusion solution was then changed to one in which LiCl was substituted for 75% of the NaCl, the K^+ concentration remaining low (0·3 mM). Fig. 5*C* was recorded after a further 6 min in this low K^+–low Na^+ solution. It can be seen that the transient inward current oscillations have become much smaller and the i_{si} magnitudes are also much reduced (i_{si} repriming is no longer oscillatory). There is also a noticeable residual very slow component of inward current after each i_{si} trace. Fig. 5*D* was taken 8 min after the preparation was returned to normal Tyrode solution. The i_{si} envelope had then returned to its normal pattern.

This parallel reduction in i_{si} and i_{TI} magnitude is shown more clearly in Fig. 6 where hyperpolarizations of one duration (600 ms) from Fig. 5*B* and *C* (i.e. in low K^+ and in low K^+–low Na^+ respectively) are shown superimposed. The outward movement of the holding current in low Na^+ (as the preparation hyperpolarizes) is

to be expected. The disappearance of the transient inward current during the pulse on changing to low Na^+ is clearly shown, as is the reduction in magnitude of the i_{si} after the pulse. The finding that both these currents diminish in low Na^+ (when Li^+ is the substitute) is evidence suggesting that they are both manifestations of Na–Ca exchange, for Li^+ cannot participate in such exchange.

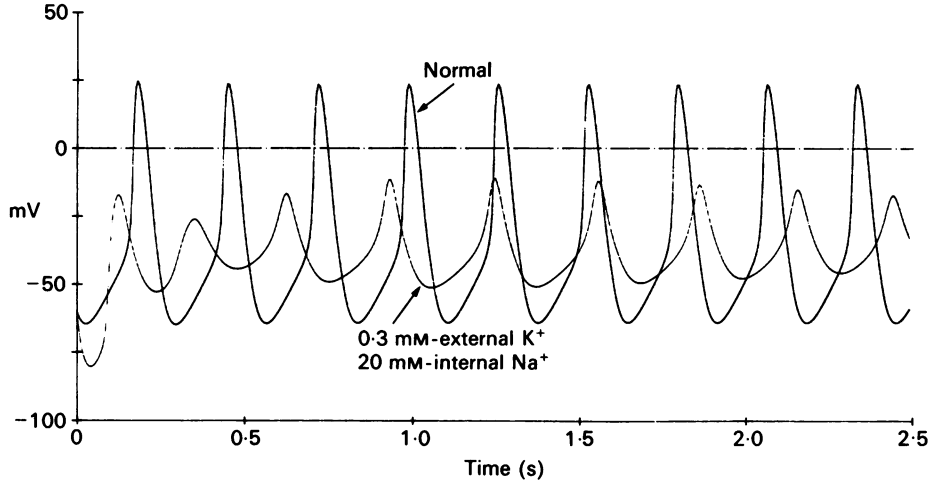

Fig. 7. Comparison between computed normal electrical activity and that produced by pump blockade in 0·3 mM-external K^+ after allowing internal Na^+ to rise to 20 mM from its normal value of 7·5 mM. (The computations shown in this and subsequent Figures were done using the peripheral sinus node option in Oxsoft Heart.)

B. Computer model results

In this section we present results obtained using the mathematical model of sino-atrial node electrical activity recently developed in this laboratory (Noble & Noble, 1984).

First, we have determined whether this model can generate the transient inward current when the activity of the Na pump is reduced. To do this we ran computations with the level of Na-pump activity reduced to between 10 and 20 % of normal. This was done by reducing the value of the Na-pump current (i_p) from its normal level of 50 nA to between 5 and 10 nA. This reduction is based on the relative level of pump activity that would be expected when extracellular K^+ concentration is reduced to 0·3 mM when 1 mM-K^+ is required for half-activation.

This reduction in pump activity is sufficient to allow the steady-state level of intracellular Na^+ to rise from 7·5 mM to 20 mM. In consequence, as a result of the activity of the Na–Ca exchange pump in the model, intracellular Ca^{2+} rises from about 100 nM to 1·4 μM. Fig. 7 shows the computed electrical activity in these conditions compared to normal electrical activity. As in the experimental records (see Fig. 1), low external K^+ and high internal Na^+ produce low voltage oscillations.

The model was then clamped at -60 mV, depolarized to $+10$ mV for 200 ms and then repolarized to various voltages between -10 and -100 mV. The results are

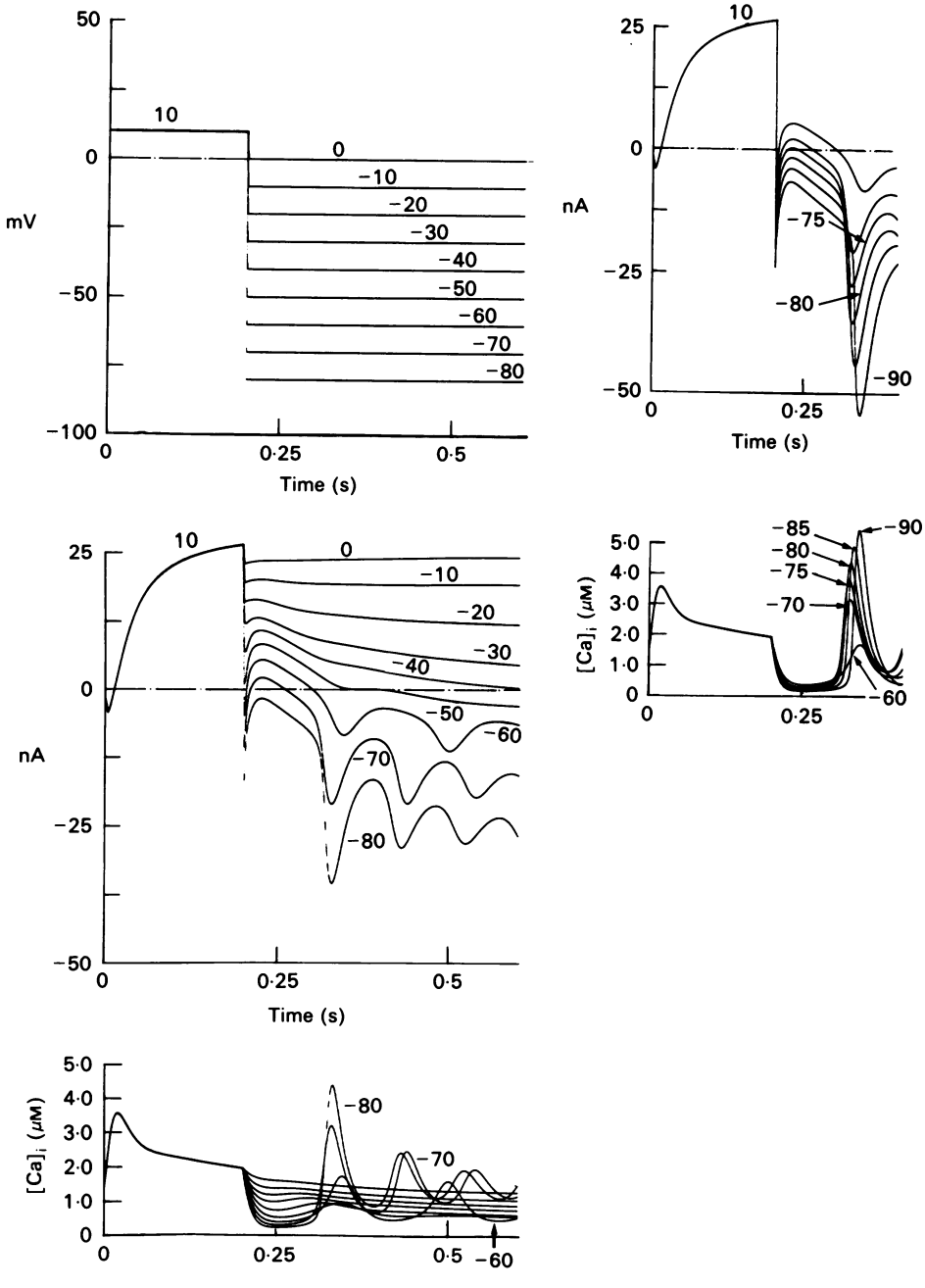

Fig. 8. For legend see opposite.

shown in Fig. 8. Clearly the model does successfully reproduce transient inward currents of the same amplitude, duration and frequency as in our experimental results. It should be remembered that these particular results were not used in setting the model up. Two other details of the experimental results are also well reproduced. First, the time-to-peak inward current is voltage dependent. This is best seen in the right-hand records which show the results at the more negative range of potentials. As the voltage is made more negative, the time-to-peak inward current first decreases and then increases. Similar phase shifts are also seen in our experimental results shown in Fig. 4. Secondly, the amplitude of the current oscillation is very strongly voltage dependent. It becomes very small as the potential approaches 0 mV. This corresponds exactly to the situation obtained experimentally in multicellular (Arlock & Katzung, 1985) and single cell (Fedida, Noble & Spindler, 1985) ventricular preparations. It does however, differ from that seen both in most Purkinje fibres and in our own sinus nodes results, where, although the current oscillations become smaller as the potential approaches 0 mV, they become larger again as the potential becomes positive. The possible reasons for this difference will be dealt with in the discussion.

The current oscillations involved in generating the transient inward current in the model are entirely attributable to oscillatory Ca^{2+} activating oscillatory variations in the Na–Ca exchange current. On this view, the related component of i_{si}, i.e. $i_{si,2}$, is also carried by the Na–Ca exchange mechanism, as postulated by Brown *et al.* (1984*a*).

The main alternative hypothesis is that both these currents are carried by Ca-activated non-specific cation channels. Can the computer model be used to distinguish between the two hypotheses?

The way we have approached this question is, first, to ask how the direction and amplitude of the current might vary during normal electrical activity on the two hypotheses. Fig. 9 shows a computer reconstruction of normal electrical activity in the model together with a computation of the time course of variation of the equilibrium potential for the Na–Ca exchange mechanism. This potential is given by eqn. (1) if the exchange process is assumed to have a stoicheiometry of 3:1:

$$E_{NaCa} = 3E_{Na} - 2E_{Ca}, \tag{1}$$

where E_{Na} and E_{Ca} are the Na and Ca equilibrium potentials respectively. By far the largest cause of the variation in E_{NaCa} will be variations in E_{Ca} during the large

Fig. 8. Oscillatory transient inward currents computed using a mathematical model of sino-atrial node cell activity. The activity of the Na–K exchange pump was reduced to 20% of normal. Intracellular Na^+ was set to 20 mM compared to a normal level of 7·5 mM. Top left: voltage-clamp steps. At time zero the model voltage was stepped from −60 mV to +10 mV. At 200 ms it was stepped back to the levels indicated. Middle left: computed net membrane currents. Bottom left: computed variations in $[Ca]_i$. Right: this shows more detail of the computed currents and $[Ca]_i$ oscillation using the same voltage protocol but with a finer grid of voltage (5 mV steps) in the second pulse. Note the phase shifts in the recorded transient inward currents. The time-to-peak inward current becomes increasingly reduced (−60 to −75 mV) and then delayed (−75 to −90 mV) as the voltage is made more negative. This behaviour is similar to that seen in Fig. 4. The associated Ca^{2+} transients are shown below and can be seen to imitate the current phase shifts as would be expected.

variations in $[Ca]_i$. The computer model reproduces these variations fairly well (see DiFrancesco & Noble, 1985). Fig. 9 shows that, with the assumptions made in this particular model, E_{NaCa} will always lie positive to the actual membrane potential, E_m. The exchange process will therefore always carry inward current and so help to maintain the action potential. By contrast, a non-specific channel would be expected to have a reversal potential near 0 mV and, therefore, to carry outward current at positive potentials.

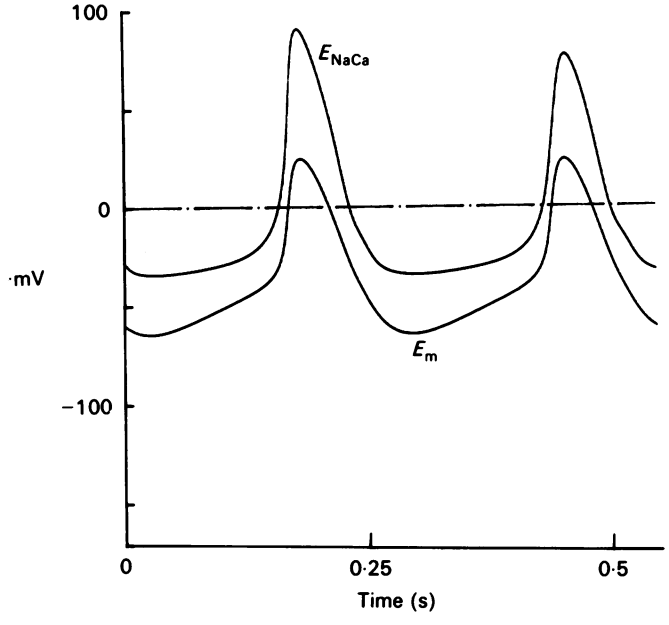

Fig. 9. Computed variation in the equilibrium potential for Na–Ca exchange, E_{NaCa}, during normal activity. Note that, in this model, E_{NaCa} is always positive to E_m so that the exchange current, i_{NaCa}, will always be negative (inward).

The computer program allows the Ca-activated non-specific channel current to be reproduced. We assumed that the channel conducts Na^+ and K^+ equally well (this in fact gives a reversal potential near -11 mV) and that the K_m for activation of the channels by $[Ca]_i$ is 1 μM. This figure is not in fact very crucial. The more important assumption is that the conductance of the channel during the Ca^{2+} transient should rise to a value sufficient to reproduce slow inward currents of the same amplitude as $i_{si,2}$, i.e. about -10 to -20 nA at -40 mV. With a K_m for Ca^{2+} activation at 1 μM, this requires a peak conductance of 1 μS in the model. The results of adding this conductance to the model are shown in Fig. 10. The effect is a clear distortion of the repolarization phase of the action potential, exactly analogous to the way in which the end-plate current (also flowing through a channel with a reversal potential near -10 mV) distorts the skeletal muscle action potential (Fatt & Katz, 1951). This kind of distortion is not seen in healthy sino-atrial node preparations, though it can be seen sometimes in deteriorating preparations or after prolonged periods of Na-pump blockade.

In the computation shown in Fig. 10 we added the non-specific channel to a model already containing the Na–Ca exchange current. It might be thought that a better comparison would be obtained by replacing the Na–Ca exchange current by the non-specific channel current. This is not possible for technical reasons. The main-

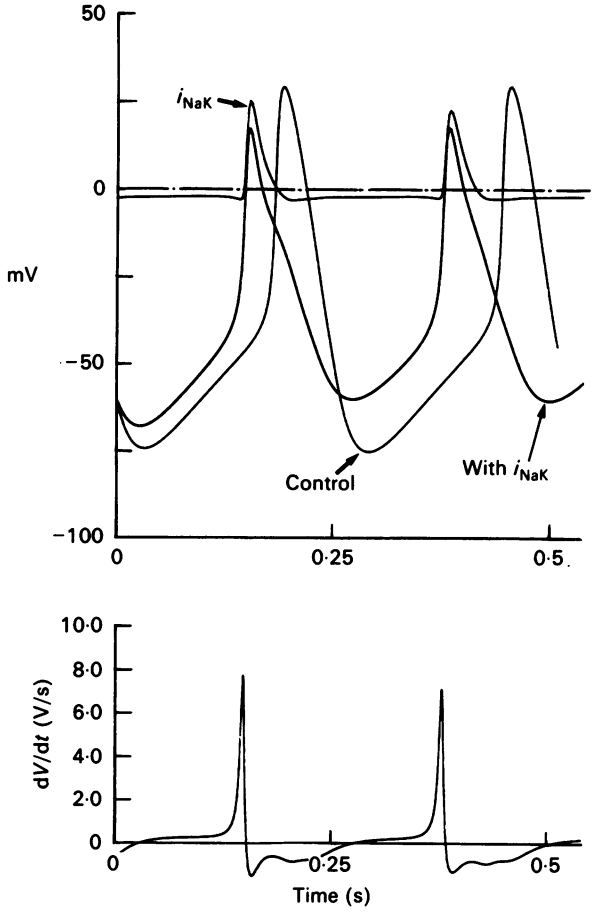

Fig. 10. Influence of a $[Ca]_i$-activated non-specific channel (i_{NaK}) on computed sino-atrial node activity. Top: control and modified action potentials and pace-maker activity. Note distortion of repolarization phase when i_{NaK} is included. The computed variation in i_{NaK} is also shown. Bottom: rate of change of voltage during computations including i_{NaK}. The distortion is now evident as a series of bumps on the negative part of each record. The conductance of the non-specific channels had to be reduced to less than 10% of the value used here for the bumps on the dV/dt plot to become negligible.

tenance of normal $[Ca]_i$ in the model is critically dependent on the activity and stoicheiometry of the Na–Ca exchange. This process cannot be removed without seriously damaging the model. Nevertheless, the computation still serves its purpose since, as noted above, the exchange current carries a current that helps to maintain the action potential. The distortion produced by the non-specific channel would be

even worse if the exchange current were abolished, though this effect would be only very moderate at positive potentials since the predicted exchange current is very small in this range.

DISCUSSION

One of the first main findings of this paper is that there is a strong resemblance between a component of the second inward current in the sino-atrial node and the transient inward current. Our interpretation of this resemblance is that, like the transient inward current, a slow component of i_{si} may be activated by intracellular Ca^{2+} rather than itself carrying Ca^{2+} into the cell. Indeed, if our preferred hypothesis for the nature of the current is correct, then it may be carrying Ca^{2+} out of the cell.

The second conclusion is that it is at present more plausible to suppose that a large part of currents like i_{TI} can be assigned to the Na–Ca exchange process rather than to current flow through Ca-activated non-specific channels. Here we will briefly review the arguments each way.

By far the strongest argument for the non-specific channel hypothesis is that, in some Purkinje fibre experiments, a clear reversal potential for i_{TI} can be found in the region of -10 mV. Initially, our own results seemed to support the Purkinje fibre work. Thus, if one pays attention to the first 300 ms of the traces shown in Fig. 4 one might well conclude that a simple case of a reversal potential had occurred. The doubts about this simple interpretation come when one inspects the record more closely. It is not then at all obvious which peak on one side of the reversal corresponds to which peak on the other side. But, more seriously, the current records are never completely flat. There is rather a voltage range (in this case from -30 mV to about 0 mV) over which the oscillations are smaller than at more extreme voltages. This is in fact what is also sometimes found in Purkinje fibres. Thus, Henning & Vereecke, (1983) found that in their Purkinje fibre experiments the current falls to nearly zero at about -30 mV and then stays very small over a 30 mV voltage range. Moreover, in ventricular preparations, both multicellular (Arlock & Katzung, 1985) and single cells (Fedida, *et al.* 1985), no reversal occurs at all since at positive potentials no oscillations are seen. This behaviour is exactly that seen in our own computer model, where the transient inward current is represented entirely as generated by the Na–Ca exchange process.

We can bring these two conclusions together by asking the question: if a slow component (what we call $i_{si,2}$) or i_{si} is generated in the same way as the transient inward current, then which hypothesis concerning its origin is most consistent with the normal electrical activity of the preparation? In Purkinje fibres, this question doesn't have much point since the plateau of the action potential is situated near the supposed reversal potential for a non-specific channel. In other regions of the heart, however, the question is important since it is evident that, as with the end-plate current and its effect on the skeletal muscle action potential, the activation of a non-specific channel current should distort the repolarization phase. Our computations (Fig. 10) confirm this expectation and put the argument into a quantitative perspective since we included sufficient non-specific channel conductance to provide an explanation for the amplitude of currents like $i_{si,2}$ and i_{TI}.

It seems therefore unlikely that all of these currents could be attributed to the

non-specific channel conductance. It should, however, be emphasized that this does not exclude the possibilities: (a) that a fairly small fraction of the current could be carried by non-specific channels or (b) that the non-specific channels could carry a large fraction of the current under abnormally large Ca^{2+} load conditions. We will deal with these possibilities in turn.

With regard to (a), our computations would suggest that not much more than 10–20 % could be carried by non-specific channels. This is about the level below which the distortion of the repolarization phase of the action potential would not be noticeable.

With regard to (b), it is of course quite possible that the two mechanisms have different sensitivities to intracellular Ca^{2+} and that, under suitable conditions the non-specific channel current could be much larger. This is in fact strongly suggested by the results of Niedergerke & Page (1982) on frog cardiac action potentials where distortion of the kind we compute in Fig. 10 is observed under conditions where the contraction (and presumably the intracellular Ca^{2+} transient) is very large.

It is interesting to note that the non-specific channel current would then dominate in determining the net reversal potential. The reason for this is that the most likely current–voltage diagram for the exchanger current would lead to the current activated by a rise in intracellular Ca^{2+} becoming negligibly small at sufficiently strong depolarizations. The precise level of voltage at which this might occur is uncertain: it depends amongst other things on how the influence of the electric field is partitioned between the forward and back reactions of the exchanger (see Noble, 1985 for a discussion of this point). The result, though, will be that the non-specific channel current would carry nearly all the current positive to its reversal potential (where the exchanger current would be expected to be very small) even if the exchanger were to carry a large fraction of the current at negative potentials. The existence of an apparently simple reversal potential (as in the original experiments of Lederer, Tsien and their colleagues) does not therefore argue against a large fraction of the transient inward current being carried by the exchanger.

Two other features of our own experimental results are worth further comment. First, replacement of Na^+ by Li^+ greatly reduces both i_{si} and i_{TI} (Fig. 6). This is readily explained by supposing that the exchanger carries the current. The argument is not however, completely conclusive since replacement of Na^+ by Li^+, by reducing the exchange activity, should eventually change intracellular Ca^{2+} and this might in turn change other Ca-activated currents. However, the simplest expectation here would be that internal Ca^{2+} should further increase in low Na^+ solutions which would be expected to have the reverse effect, i.e. to increase the current carried by Ca-activated non-specific channels.

The second feature that we wish to draw attention to is the fact that, in addition to i_{si} itself, our results show the presence of an extremely slow inward current tail lasting several hundred milliseconds (see Fig. 5). This result was noted previously by us (Brown *et al.* 1984*a*) and we also showed that our computer model could reproduce it as a low level of exchange current flowing while intracellular Ca is slowly returned to its basal level after the main fall (attributable to sarcoplasmic reticulum uptake) has occurred. It is interesting to note that this phase of the current is much more pronounced in low Na^+ (Li^+ replacement) solutions, as is very clear in Fig. 5*C*. This

might be expected if the background level of free Ca^{2+} is much higher since there would then be more Ca^{2+} to be eliminated to achieve the complete return to the higher basal level.

Finally, our results reveal an interesting and potentially very valuable feature of the computer model. This is that, without any further assumptions than those required to reproduce normal electrical activity in the sino-atrial node, the model is capable, in response to reduced Na-pump activity, of generating transient inward currents. As Fig. 8 shows, this is because, at high levels of free intracellular Ca^{2+} the Ca-induced Ca^{2+} release process represented in the model becomes oscillatory. DiFrancesco, D., Hart, G. & Noble, D. (unpublished; see Noble, 1984, Fig. 16) have also shown that this is true for the Purkinje fibre version of the model.

While the model succeeds in reproducing transient inward currents when the Na–K exchange pump activity is reduced, it should be noted that the voltage range at which the oscillations occur is more negative than in the experimental results. This range depends on the range over which Ca release is oscillatory, which is sensitive to parameters in the model that are not well defined experimentally. Hopefully, this feature can be improved as more quantitative information on the Ca^{2+} dependence of the release process is made available.

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