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A model of sino-atrial node electrical activity based on a modification of the DiFrancesco–Noble (1984) equations

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DiFrancesco & Noble's (1984) equations (*Phil. Trans. R. Soc. Lond.* B (in the press.)) have been modified to apply to the mammalian sino-atrial node. The modifications are based on recent experimental work. The modified equations successfully reproduce action potential and pacemaker activity in the node. Slightly different versions have been developed for peripheral regions that show a maximum diastolic potential near −75 mV and for central regions that do not hyperpolarize beyond −60 to −65 mV.

Variations in extracellular potassium influence the frequency of pacemaker activity in the s.a. node model very much less than they do in the Purkinje fibre model. This corresponds well to the experimental observation that the node is less sensitive to external [K] than are Purkinje fibres.

Activation of the Na–K exchange pump in the model by increasing intracellular sodium can suppress pacemaker activity. This phenomenon may contribute to the mechanism of overdrive suppression.

Introduction

The computer model we shall describe here is a version of the cardiac cell model developed by DiFrancesco & Noble (1982, 1984). This model differs quite radically from previous models of cardiac electrical activity, including those developed recently for the s.a. node (e.g., Yanagihara *et al.* 1980; Irisawa & Yanagihara 1980; Irisawa & Noma 1982). The main difference lies in the fact that, instead of restricting the description of membrane ionic current to the classical gated and background conductances, the new model fully incorporates the currents generated by the Na–K and Na–Ca exchange processes and reconstructs the variations in intracellular and extracellular ion concentrations.

Our approach in modifying the model for the s.a. node has been to use the same equations as DiFrancesco & Noble (1984) with parameters appropriate to the s.a. node except where specific information exists on the s.a. node that requires the equations to be changed.

Nomenclature

The definition of symbols may be found in DiFrancesco & Noble (1984). The phrase 'total second inward current' will be used to refer to the overall inward current generated by the voltage-gated calcium channel and by Ca-dependent

inward currents, such as the sodium–calcium exchange current. The phrase 'gated Ca channel' will be used to refer to the voltage-gated calcium channel only.

Description of model

(*a*) *Geometry of the preparation and extracellular* K^+ *diffusion*

Voltage clamp experiments are carried out on preparations that have been cut down and ligatured to a disc whose dimensions are about $0.2 \times 0.2 \times 0.1$ mm^3. Some of the regions near the edges must contain damaged or disconnected cells so that the active preparation will be somewhat smaller in size. We have assumed the total volume of the active preparation to be 80% of the dissected preparation. The extracellular space was assumed to occupy 10% of the active volume. For most computations we used the three compartment model to describe the variations in extracellular [K] (see DiFrancesco & Noble (1984), equation (63)). For some computations (for example, those reproducing the extracellular [K] measurements of Maylie *et al.* (1981), see Brown *et al.* 1984*b*) we used the full diffusion equations for cylindrical or spherical preparations (DiFrancesco & Noble (1984), equations (59) or (62)). For the three compartment model the time constant for diffusion of K^+ between the cleft and bulk space was usually set to 1 s. For the full diffusion computations we used either the free solution K^+ diffusion coefficient or this value reduced by 50% to represent any restriction of diffusion in the extracellular space. The results described in this and the accompanying papers (Brown *et al.* 1984*a*, *b*) did not depend critically on the assumptions made on this point.

The membrane capacitance was set to 0.006 μF. This value is based on the value of 0.0059 μF given by Noma & Irisawa (1976), who also show that this total capacitance is consistent with a specific membrane capacitance of 1 μF cm^{-2} when account is taken of the size, number and shape of the cells in a typical preparation.

(*b*) *Calcium* (*second inward*) *current and sodium–calcium exchange current*

The DiFrancesco–Noble model distinguishes between the fast gated second inward current, $i_{Ca,f}$, and slower inward current carried by other mechanisms such as the Na–Ca exchange process, i_{NaCa}. This has turned out to be a crucial distinction in the case of the s.a. node (Brown *et al.* 1984*a*) and therefore forms an important part of the s.a. node model. The total 'second inward' current recorded experimentally in the s.a. node is found to be the sum of at least two components (Brown *et al.* 1982, 1984*a*). In the model we have assumed these components to be the voltage-dependent gated current and the Na–Ca exchange current. Other Ca-activated currents, inward or outward, may also be involved (Siegelbaum & Tsien 1980; Colqhoun *et al.* 1981). It is not therefore possible to use the experimental voltage clamp data on the multicellular s.a. node preparation to determine uniquely the kinetics of the voltage-gated component. The DiFrancesco–Noble model uses kinetics based on the very fast current records obtained recently from voltage clamp of single cells. Although the speed of the equations for the voltage-gated component is not directly based on the s.a. node work, the steady state activation and inactivation curves are nevertheless very similar to the 'dynamic' curves found experimentally in this laboratory (Brown *et al.* 1984*a*). Such a correspondence would not be unexpected if the other

components depend on the degree of activation of the fast voltage-gated component. This is certainly the case in the DiFrancesco–Noble model since the slow component is attributed to electrogenic Na–Ca exchange in response to Ca release induced by calcium entering via the gated channels. Moreover, we shall show elsewhere (Brown *et al.* 1984*a*, *b*) that the model does successfully reproduce the fast and slow components of i_{si} in our experimental work.

Brown *et al.* (1982, 1984*a*) have shown the presence of Ca-entry induced inactivation of the Ca current in the s.a. node. This feature is included in the DiFrancesco–Noble equations. For the s.a. node results, we found that a good simulation could be obtained by setting the time constant (τ_{f2}) for recovery from inactivation to 0.1 s, with the constant ($K_{m,f2}$) determining the level of $[Ca]_i$ at which half inactivation occurs in the steady state set to 1 μM.

The value for the permeability factor, P_{si}, was set to 12. This generates a peak current of about −70 nA on depolarizing from −50 mV to 0 mV, which is similar to the maximum current recorded experimentally. Some experiments show considerably less current, which may simply be due to series resistance problems (cf. Noble & Powell 1983). The extent to which the equations can reproduce the properties, and particularly the slow components, of i_{si} in the s.a. node will be investigated in Brown *et al.* (1984*a*). As in the DiFrancesco–Noble equations, two alternative formulations have been used for i_{NaCa}. The simplest (DiFrancesco & Noble (1984), equation (22)) makes the current a sinh function of the total energy gradient, including the ionic gradients and membrane potential. For this case we used a value for k_{NaCa} (the scaling factor for i_{NaCa}) of 0.2. This generates 'slow' inward currents similar to those recorded experimentally (see Brown *et al.* (1982) for an example using this version). The more realistic model uses DiFrancesco & Noble's (1984) equation (26) which is likely to reproduce better the dependence of i_{NaCa} on intracellular calcium ions. This is important during natural electrical activity. For this version of the model (which is the version used in most of the applications described by Brown *et al.* 1984*a*, *b*) two constants are required. The scaling factor, k_{NaCa}, was set to 0.02 and the 'denominator' factor d_{NaCa} was set to 0.001. Again the criterion used was whether the model successfully reproduces the slower components of the total 'i_{si}' current. In both cases the stoichiometry was set to 3:1 (Na:Ca). These values give a diastolic free intracellular calcium concentration in the range 50–100 nM.

(*c*) *Delayed* K^+ *current*

The delayed K^+ current is activated in a similar voltage range in Purkinje fibres (Noble & Tsien 1969) and in s.a. node (DiFrancesco *et al.* 1979; Yanagihara *et al.* 1980), but the kinetics are significantly faster in the s.a. node. We therefore replaced the DiFrancesco–Noble equations with equations based on the s.a. node results. Our equations are

$$dx/dt = \alpha_x(1-x)-\beta_x x, \tag{1}$$

$$\alpha_x = 0.5(E+22)/(1-\exp(-(E+22)/5), \tag{2}$$

$$\beta_x = 0.178(E+22)/(\exp((E+22)/15)-1), \tag{3}$$

$$i_K = \bar{i}_K x. \tag{4}$$

These equations give an activation curve that is somewhat steeper than that of DiFrancesco *et al.* (1979). This is because in the experiments in this laboratory it is usually found that very little or no i_K is activated in the steady state at -35 mV. (When computing the effects of abolishing the decay of i_K (see Brown *et al.* 1984*b*) dx/dt has been set to zero.)

The function for the fully activated current, i_K, used in the DiFrancesco–Noble model is very similar to that found in the s.a. node. It shows inward-going rectification but with no negative slope region and no cross-over at different external [K]. We have therefore used the DiFrancesco–Noble function. The maximum outward current, $i_{K,m}$, was set to 30 nA at $[K]_c = 3$ mM (which is similar to that recorded experimentally in this laboratory and by DiFrancesco *et al.* (1979)). The advantage of using the DiFrancesco–Noble equation for i_K rather than, for example, that of Yanagihara *et al.* (1980) is that the DiFrancesco–Noble function represents the dependence of the current on external [K].

(*d*) *The background* K^+ *current*

We used the same function as in the DiFrancesco–Noble equations but with the maximum value for g_{K1} greatly reduced to 10 μS. Using the value of $i_{K,m}$ as a comparison, this represents only 5 % of the value used in the Purkinje fibre model. Using i_f as the basis for comparison, the fraction is even smaller. It is difficult to estimate what the value should be experimentally since such a small current cannot easily be distinguished from instantaneous jumps of current due to other mechanisms. All we can say for certain is that i_{K1} should be sufficiently small for the K^+ depletion process during hyperpolarization to be insufficient to mask the activation of i_f since, in s.a. node, i_f does not display the properties that led to its identification as i_{K2} in Purkinje fibres (Noble & Tsien 1968): that is, it never shows a pseudoreversal potential accompanying strong hyperpolarization (see DiFrancesco & Noble 1984). In this respect, the s.a. node resembles the Purkinje fibre when i_{K1} has been greatly reduced by adding Ba^{2+} ions to the bathing medium (DiFrancesco 1981).

(*e*) *The hyperpolarizing-activated current*

This current was first recorded in Purkinje fibres (Vassalle 1966) and later identified and analysed as i_{K2} (Noble & Tsien 1968). The current in the s.a. node whose switching range and kinetics resemble those for i_{K2} is the hyperpolarizing-activated current called i_f (Brown *et al.* 1979) in the DiFrancesco–Noble (1984) model and i_h by other workers (Noma *et al.* 1977). More recently, i_{K2} in the Purkinje fibre has been reinterpreted as a hyperpolarizing-activated current (DiFrancesco 1981*a*, *b*, 1982). The DiFrancesco–Noble equations were in fact originally developed to explore the theoretical consequences of this reinterpretation (DiFrancesco & Noble 1980, 1982).

While it is easy to record i_f during hyperpolarizations in the s.a. node (indeed, negative to about -70 mV i_f dominates the current record) it is not easy to construct reliable activation curves. The reason for this is that the deactivation of i_f on return to holding potentials in the range -30 to -50 mV is fast and i_f becomes readily confused with other current changes. The activation curves given

by Yanagihara & Irisawa (1980*a*) occupy a very similar switching range to that in the DiFrancesco–Noble equations and we found that we could obtain a good simulation of the current records by using the DiFrancesco–Noble equations with the kinetics speeded up by a factor of 2 (that is, about the same factor by which we have speeded up the kinetics of i_K) to give a peak time constant of about 3 s instead of 6 s as in the DiFrancesco–Noble model. The peak values of $g_{f,Na}$ and $g_{f,K}$ were set to 6 μS. This gives current amplitudes similar to those recorded experimentally and the reversal potential for i_f then lies midway between E_{Na} and E_K, which is about −20 mV (Yanagihara & Irisawa 1980, figures 9 and 10; Kimura 1982).

(*f*) *The sodium–potassium exchange pump*

Noma & Irisawa (1974) observed a substantial hyperpolarization and suppression of pacemaker activity in the s.a. node when K^+ ions were readmitted to the bathing solution after a period of superfusion with K-free solution. This effect was abolished by ouabain. Similar results are obtained in Purkinje fibres and, when the voltage clamp technique is used, the currents recorded may be used to estimate the current carried by the Na–K exchange pump. It was data obtained in this way that was used to formulate the Na–K pump equations in the DiFrancesco–Noble model (see Gadsby & Cranefield 1979; Gadsby 1980; Eisner & Lederer 1980). Recently, Kurachi *et al.* (1981*a*, *b*) have used exactly the same approach to estimate the pump current in the a.v. node. We have used the DiFrancesco–Noble equations with the maximum pump current, i_p, set to 50 nA. This is sufficient to allow the internal and external ion concentrations to be maintained in a steady state during normal repetitive activity and to generate current changes during pump reactivation similar to those recorded experimentally.

(*g*) *The sodium current*

A small TTX-sensitive current is found in the s.a. node (Kreitner 1978; Noma *et al.* 1977). Its role though is clearly not important compared to that in the Purkinje fibre. For completeness we have included a small component in the model by setting the value of g_{Na} to 1.25 μS in the DiFrancesco–Noble equations.

(*h*) *Calcium storage and release*

We used the DiFrancesco–Noble equations for Ca uptake and release. Maylie *et al.* (1981) measured tension during repetitive activity in the s.a. node and found that relaxation starts before the end of repolarization (Maylie *et al.* 1981, figure 1). The intracellular $[Ca]_i$ transient must therefore reach its peak quite early in the action potential and be declining to relatively small values during repolarization. We have adjusted the parameters in the DiFrancesco–Noble equations to give this result. The parameters used to do this were: (i) the uptake time constant was set to 5 ms; (ii) the repriming time constant was set to 100 ms (that is, considerably faster than in Purkinje fibres); (iii) $K_{m,Ca}$ (the binding constant for the Ca-induced Ca release) was set to 0.001 mM and (iv) the release process was assumed to be second order with $r = 2$.

Of these assumptions, by far the most important is the value of r. DiFrancesco *et al.* (1983) found that r must be at least 2 to generate oscillatory release of calcium

of the kind required to account for the oscillatory t.i. current in Purkinje fibres. Similarly we found $r = 2$ readily allows the very slow component of 'total slow inward current' (see Brown *et al.* 1982; Brown *et al.* 1982, 1984*a*) to be reproduced. We think this current is analogous to $i_{t.i.}$ in Purkinje fibres, which is also thought to be a slow inward current generated by changes in intracellular calcium concentration.

(*i*) *Ion concentrations*

$[K]_i$ was set to 140 mM, $[Na]_i$ was usually set to 7.5 mM and $[K]_b$ was usually set to 3 mM. $[Ca]_o$ was set to 2 mM.

(*j*) *Other parameters*

There are a few other parameters in the DiFrancesco–Noble equations that remain to be set: g_{to} was set to 0 since it has not been found in the s.a. node; $g_{b,Na}$ was set to 0.07 μS and $g_{b,Ca}$ was set to 0.01 μS. The value of $g_{b,Na}$ was chosen as that which allowed a maximum diastolic potential between about −70 and −55 mV to be achieved during pacemaker activity. The value of $g_{b,Ca}$ was chosen to give a diastolic level of free calcium in the range of 0.0005 mM.

Results and discussion

Most of the experimental applications of the model developed here from the DiFrancesco–Noble equations will be described in two accompanying papers (Brown *et al.* 1984*a*, *b*). Discussion of these applications and the justification for some of the model parameters will therefore be deferred to these papers. Here we shall draw attention to a few general points, and to two specific properties of the equations which will not be dealt with in those papers.

First, there is some diversity in s.a. node electrical activity. Peripheral regions of the node and the ring bundle can show pacemaker activity with maximum diastolic potentials of up to −75 mV. Our 'standard' model reproduces this situation. Central cells, however, tend to have much lower maximum diastolic potentials, around −55 to −65 mV. There are various possible ways in which the standard model can be adjusted to give a less negative maximum diastolic potential. These involve either reducing i_K, increasing a background inward current by increasing $g_{b,Na}$ or changing the parameters determining i_{NaCa}.

Figure 1 shows our 'standard' model and an example of a lower amplitude model. In each case we have also plotted dV/dt. Maximum values of around 10 V s^{-1} are obtained, which are similar to typical experimental values.

It may be noticed that, in both results, there is a very slight wobble on the negative phase of the dV/dt plot, corresponding to the repolarization phase. This is so small that it is not evident in the repolarization phase itself. In an earlier version of the equations, which lacked the Ca-entry dependent inactivation of the calcium current, we found a much more significant bump on the repolarization phase, attributable to the calcium 'window' current. Ca-entry dependent inactivation almost entirely eliminates this problem.

One of the striking differences between pacemaker activity in Purkinje fibres and s.a. node pacemaker activity is that, whereas the former is extremely sensitive to

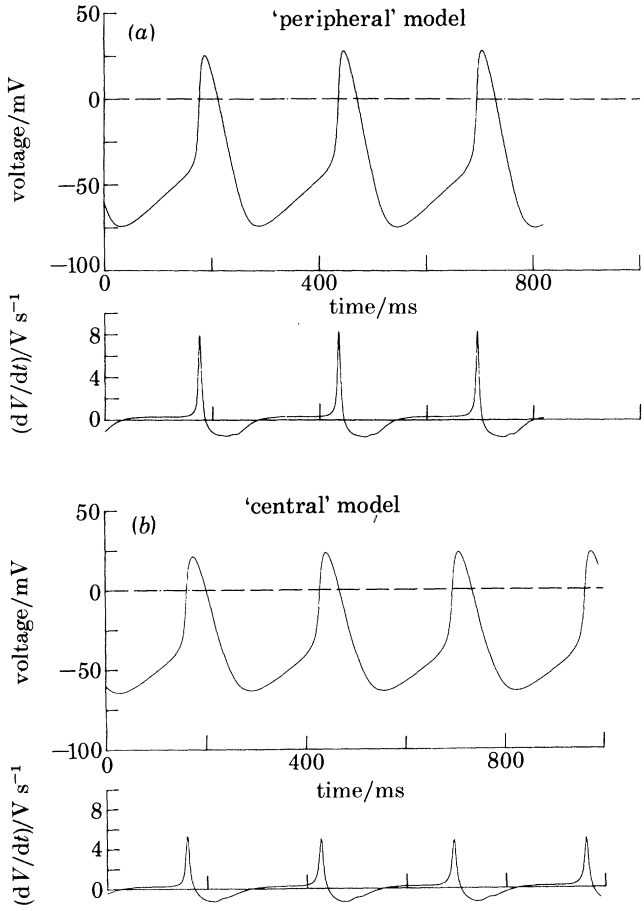

FIGURE 1. (*a*) Standard 'peripheral cell' model. Top record shows computed voltage changes. The bottom record shows the rate of voltage change. (*b*) Standard 'central' cell model. This differs from the peripheral cell model in that P_{si} is reduced to 8 and $i_{K,max}$ is reduced to 20.

extracellular potassium ions, the latter is fairly insensitive. Thus, in Purkinje fibres, a change in $[K]_b$ from 4 to 5.4 mM can be sufficient at least to greatly slow and often to abolish pacemaker activity completely. The natural cardiac rhythm generated by the s.a. node is only moderately sensitive to such changes. Since the s.a. model developed here is based on equations originally developed for the Purkinje fibre, it is important to check that the changes made do allow this striking difference to be reproduced correctly. Figure 2 shows that this is indeed the case. Here we show superimposed computations of central cell activity at $[K]_b$ concentrations of 2, 3 and 6 mM. In agreement with experimental findings, the frequency change is only moderate. This is dramatically different from the effect of such a reduction in $[K]_b$ on Purkinje fibre activity (see Vassalle (1965) for experimental records and DiFrancesco & Noble (1984), figure 7, for the behaviour of the DiFrancesco–Noble equations). The reason for this difference lies in the fact

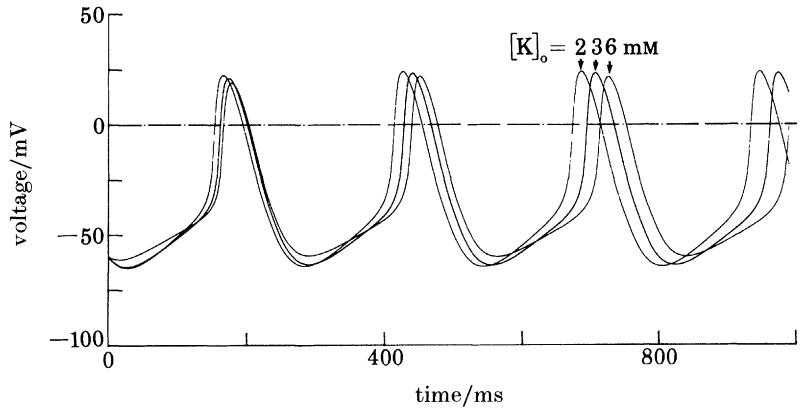

FIGURE 2. Influence of variations in extracellular [K] on computed central cell activity. $[K]_b$ was set to 2, 3 or 6 mM.

that the inward rectifier, i_{K1}, is relatively unimportant in the s.a. node, but is very significant in Purkinje fibres. It is largely the effect of external K^+ ions on i_{K1} that is responsible for the extreme sensitivity of Purkinje fibre pacemaker activity to external potassium concentration.

It should be emphasized that the insensitivity of pacemaker activity in the s.a. node to direct effects of external potassium does not preclude sensitivity to indirect effects. In very low K^+ solutions, for example, the Na–K exchange pump will be inhibited and Noma & Irisawa (1974) have shown that the reactivation of the pump after several minutes in a K^+-free solution can produce a current that is sufficient to suppress pacemaker activity. This may also be the basis, in part, of the phenomenon of overdrive suppression. In figure 3 we show that a sufficiently large increase in pump activity (in this case by allowing $[Na]_i$ to increase to 30 mM) will abolish pacemaker activity in the s.a. node model.

Finally it is worth emphasizing that, like the main DiFrancesco–Noble model, we see the present formulation as a framework for future development as much

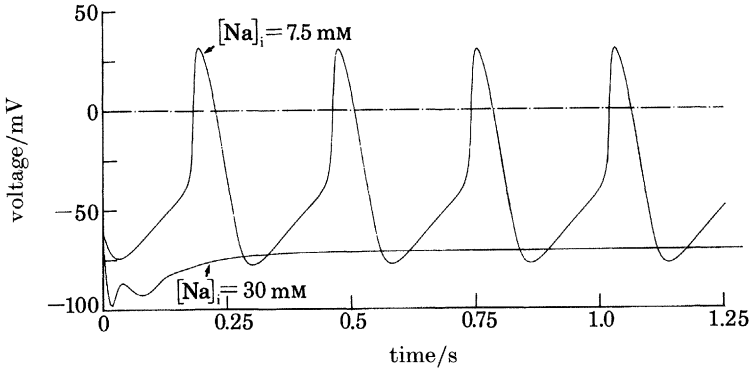

FIGURE 3. Influence of activating Na–K exchange pump by increasing $[Na]_i$ to 30 mM. Pacemaker activity is abolished.

as a working hypothesis to account for present results. The range of experimental data is now such that it is essential for realistic modelling of the electrical properties of the node to take account of the existence of ionic pumps, concentration changes and tissue geometry. The need for such complexity will be clear from the experimental data described in Brown *et al.* (1984*a*, *b*). The DiFrancesco–Noble model and the s.a. node development of it described here is the first model that attempts to interpret fully the electrical activity at this level of complexity. The main reason for which we have developed it is that models incorporating only voltage-dependent conductances fail completely to reproduce many of the experimental results obtained in voltage clamp work on the s.a. node including those from this laboratory.

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