Journal of Physiology (1990), **429**, *pp.* 401–409
With 4 figures
Printed in Great Britain

PACEMAKING IN RABBIT ISOLATED SINO-ATRIAL NODE CELLS DURING Cs^+ BLOCK OF THE HYPERPOLARIZATION-ACTIVATED CURRENT i_f

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(*Received 5 February 1990*)

SUMMARY

1. Experiments have been carried out using the whole-cell patch clamp technique to investigate how the spontaneous pacemaker activity of rabbit isolated sino-atrial (SA) node cells is affected by the block of the hyperpolarization-activated current i_f by 1 and 2 mM-caesium.

2. Two millimolar caesium reduced the amount of i_f activated at −90 mV to less than 10% of the control value. In the pacemaking range of SA node cells, i_f was often completely blocked by this concentration of Cs^+.

3. Two millimolar caesium slowed but did not arrest spontaneous pacemaking in isolated SA node cells. In freely beating non-patched cells, 2 mM-CsCl caused a 30% reduction in rate of beating, indicating that in all cells observed i_f was normally contributing to pacemaking.

4. No increase in instantaneous inward current was seen in response to hyperpolarizing voltage clamp pulses from a holding potential of −40 mV when 1 or 2 mM-CsCl was applied to SA node cells. These concentrations of Cs^+ do not therefore induce an 'extra' inward current.

5. Neither the inward calcium current (i_{Ca}) nor the outward potassium current (i_K) showed changes which could be attributed to Cs^+ application.

6. Since spontaneous pacemaking continues during Cs^+ block of i_f while other membrane currents show no Cs^+-induced changes which could account for this, these experiments provide strong, though indirect, evidence for the presence in SA node of a time-independent background current, i_b, which will contribute to a mode of pacemaking controlled by the decay of potassium conductance (g_K). The nature of this inward current has yet to be clarified.

7. The results strongly suggest that the hyperpolarization-activated current i_f normally makes an important contribution to the pacemaker depolarization of all SA node cells.

INTRODUCTION

The extent of the contribution of the hyperpolarization-activated current i_f to pacemaking in sino-atrial node is still vigorously disputed (DiFranceso & Noble, 1989; Hagiwara & Irisawa, 1989; Hartzell, 1989). The properties of this current fit it well for pacemaking: it is an inward current increasingly activated the more the

cell is hyperpolarized and it is rapidly deactivated at positive potentials (as during each action potential). DiFrancesco & Noble (1989) have developed a partial SA node model in which all the initial part of the pacemaker depolarization up to the threshold of the calcium current i_{Ca} is attributed to i_f. In experimental recordings from SA node cells, however, there is often rather little i_f activated within the relatively positive pacing range of SA node cells (−65 to −45 mV) although it must be borne in mind that the high input impedance of these cells (about 1 GΩ; see Denyer & Browns 1990) means that just a very few picoamps of current will be sufficient to have a significant depolarizing effect on their membranes.

Another mechanism proposed for the SA node cell pacemaker depolarization is one which is controlled by the decay of the outward potassium conductance g_K activated during the preceding action potential. Decay of g_K could not by itself cause the membrane to depolarize; it must be superimposed on a constant inward 'background' current i_b, the nature of which is still uncertain.

The single-cell SA node model of Noble, DiFrancesco & Denyer (1989) is based on the g_K decay hypothesis: g_K decay superimposed on a constant inward background current produces the net inward current change generating the pacemaker potential. In this model the background current is a background 'leak' current carried mainly by sodium ions but this could well be too simple a view. Other currents contribute to the net current during the pacemaker depolarization. These include the sodium–potassium pump current i_{NaK}, sodium–calcium exchange current i_{NaCa}, the hyperpolarization-activated current i_f, and the calcium current i_{Ca}, all of which are activated within the pacemaker range (see also Discussion).

Doerr, Denger & Trautwein (1989) have proposed that T- and L-type calcium currents contribute significantly to the pacemaker potential from its onset. The cells they use are derived from the central area of the node and have maximum diastolic potentials (MDPs) of only −50 mV which is at or near the threshold level of inward calcium current. The cells we have used in this study had an average MDP of −65 mV, a potential well negative to gated Ca^{2+} current threshold (see Denyer & Brown, 1990). Although it is important to establish the mode of pacemaking in the central area of the node, it is also clear that SA nodal cells with MDPs more negative than −50 mV can pace and do so persistently. The question addressed here is whether i_f is the only current contributing to pacemaking between −65 and −50 mV or whether another current system is also involved.

If it can be shown that spontaneous pacemaking continues when the hyperpolarization-activated current i_f is blocked in the pacing range of SA nodal cells by a substance which does not significantly alter other membrane currents, then this will provide strong indirect evidence for the contribution of inward background current to SA nodal pacemaking.

The blocking substance we have used is caesium (1 and 2 mM-CsCl). Most previous investigations of the effects of Cs^+ on SA node have used multicellular preparations (Brown, DiFrancesco, Kimura & Noble, 1981; Noma, Morad & Irisawa, 1983) and/or used only 1 mM-Cs^+, a concentration which is not sufficient to block i_f completely (van Ginneken, 1987). In this study, we have carefully examined the effects of concentrations of 1 and 2 mM-Cs^+ on the spontaneous pacemaking of single SA node cells and on i_f and other membrane currents.

METHODS

The cell isolation procedure, the recording method (whole-cell patch clamp technique) and the solutions used have been described in Denyer & Brown (1990). In the present experiments Cs^+ was added as CsCl to normal Tyrode solution to give concentrations of 1 or 2 mM. Some recordings were made using the permeabilized patch technique described by Horn & Marty (1988). For these, nystatin (0·14 mg/ml) was included in the pipette solution.

The estimates of spontaneous beating rates given in this paper were made by observing beating cells under the microscope and counting the time for thirty beats with the help of a mechanical counter and a stop-watch. Only regularly beating cells were counted, each reading being the average of three counts and care was taken to control the temperature.

RESULTS

Activation of the hyperpolarization-activated current i_f is very evident when hyperpolarizing voltage clamp pulses are given to an SA node cell from a holding potential of −40 mV, as is shown in Figs 8–10 of Denyer & Brown, 1990. Those figures show that there is relatively little i_f activated within the usual SA nodal cell pacemaker range (−65 to −45 mV) even by the end of the 800 ms clamp hyperpolarizations given; the bulk of this current is activated at more negative potentials.

Figure 1 shows the i_f activated in an isolated SA node cell (in response to clamp hyperpolarizations from −40 mV in 5 mV steps down to −90 mV) firstly in Tyrode solution containing 1 mM-Cs^+ (Fig. 1*A*), then on return to normal Tyrode solution (Fig. 1*B*), then in 2 mM-Cs^+ Tyrode (Fig. 1*C*), and finally on recovery in normal Tyrode solution once more (Fig. 1*D*). In 1 mM-Cs^+ i_f was partially blocked and in 2 mM-Cs^+ the block of i_f was almost complete, particularly in the SA nodal pacemaker range (−65 to −45 mV). There was full recovery of i_f on return to normal Tyrode solution.

Figure 2 shows superimposed records of the spontaneous activity of an isolated SA node cell in normal Tyrode solution and in Tyrode solution to which 2 mM-Cs^+ had been added. Although there was a slowing of the rate, spontaneous activity continued in 2 mM-Cs^+.

A possible difficulty in interpreting this result is that in whole-cell patch clamped cells, current leak through the seal between the cell membrane and the electrode could result in an inward current which could be contributing to pacemaker activity. We looked carefully therefore at the effect of 2 mM-Cs^+ on the spontaneous rate of freely beating SA node cells to which no electrode had been applied. In thirteen cells, the average rate of spontaneous beating before the application of Cs^+ was 166 beats/min (S.E.M ±4·25). The addition of 2 mM-CsCl to the superfusate caused a reduction in average beating rate to 118 beats/min (S.E.M ±7·16). On return to normal Tyrode solution the average beating rate recovered to 159 beats/min (S.E.M ±4·77). In every cell tested, Cs^+ caused a marked reduction in spontaneous frequency but in no case did the beating cease; the recovery of beating frequency when Cs^+ was removed was almost complete.

If a 'seal leak' current does contribute to the pacing of whole-cell patched cells, the reduction in beating frequency caused by Cs^+ in unpatched cells will be greater than

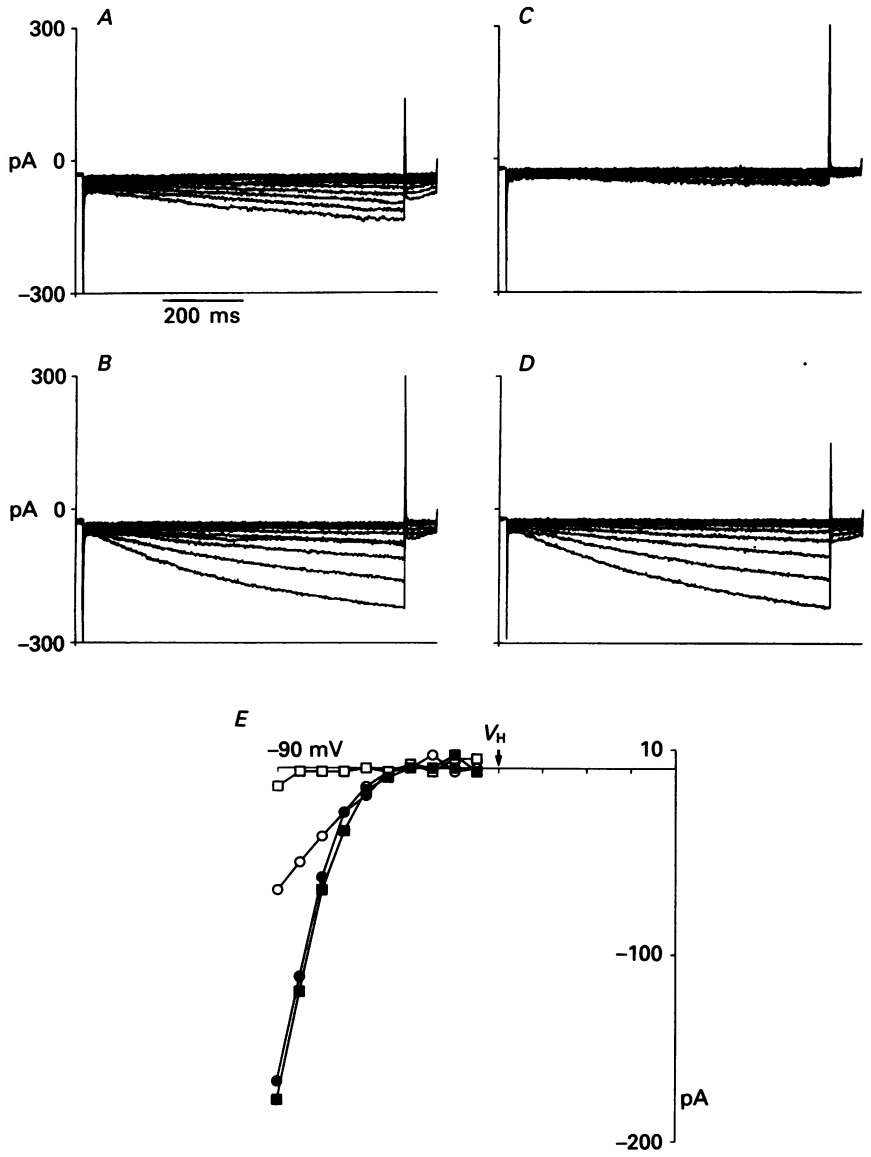

Fig. 1. *A–D*, hyperpolarization-activated current i_f recorded from an isolated SA node cell in response to 800 ms voltage clamp hyperpolarizations from a holding potential (V_H) of −40 mV in 5 mV steps down to −90 mV. *A*, in Tyrode solution containing 1 mM-CsCl. *B*, after recovery in normal Tyrode solution. *C*, in Tyrode solution with 2 mM-CsCl. *D*, after recovery in normal Tyrode solution again. *E*, isochronal activation curves for i_f in *A–D* plotted from current magnitudes at the end of each pulse. Open symbols: in Cs^+ Tyrode solution, 1 mM (○) and 2 mM (□). Filled symbols: in normal Tyrode solution.

in patched ones. We looked for evidence of this but failed to find any large or consistent differences (but see Discussion).

To check whether Cs^+, in addition to blocking i_f, causes changes in other membrane currents which could account for the continued spontaneous beating, we first looked

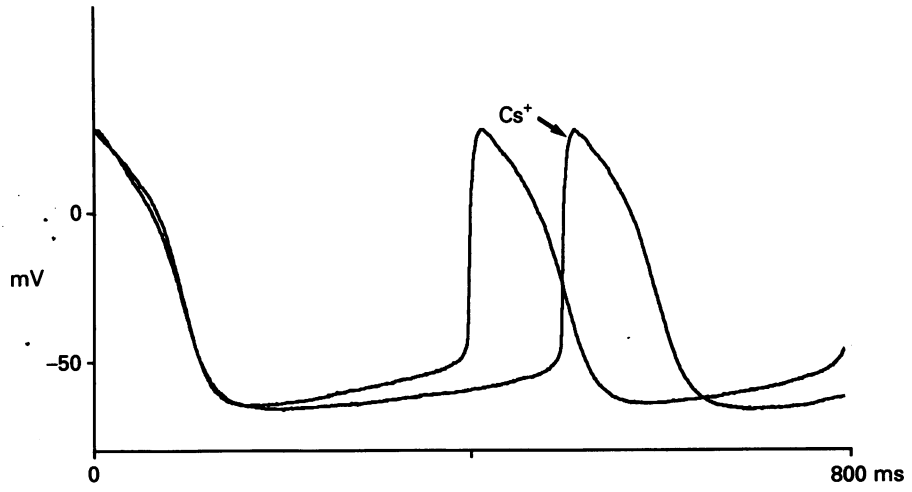

Fig. 2. Whole-cell nystatin-permeabilized patch clamp recording of spontaneous activity in an isolated SA node cell before and during superfusion with Tyrode solution containing 2 mM-CsCl.

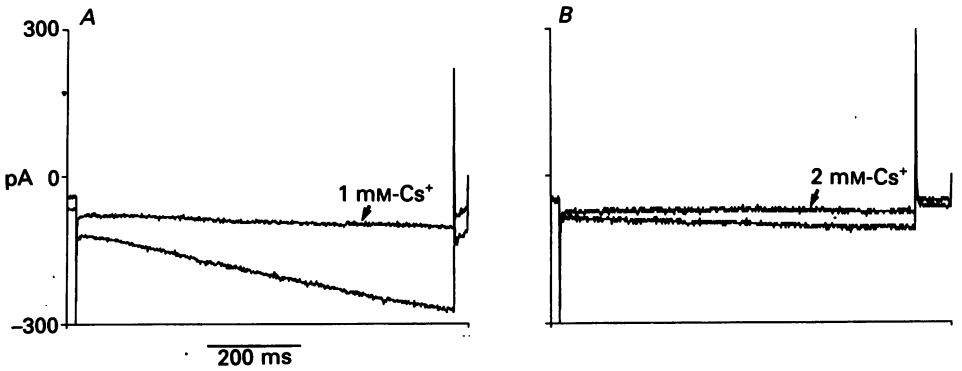

Fig. 3. Hyperpolarization-activated current recorded in two different SA node cells in response to voltage clamp pulses from −40 to −70 mV before and during superfusion with Tyrode solution containing 1 mM (*A*) and 2 mM (*B*) CsCl. Note that while Cs^+ blocks i_f during the pulse, it causes no increase in the inward current jump at the start of the pulse. In *A*, there was an outward shift of both holding current and initial jump in Cs^+ indicating that in this cell some i_f was already activated at −40 mV.

carefully to see whether any increase in instantaneous inward current could be detected when Cs^+ was applied.

Figure 3 shows the currents recorded in two SA node cells in response to 800 ms hyperpolarizing voltage clamp pulses to −70 mV from a holding potential of −40 mV. Such pulses elicit an initial inward current jump which in normal Tyrode is followed by gradual activation of i_f during the pulse. Cs^+ was applied to both cells: 1 mM-Cs^+ to the cell in Fig. 3*A* and 2 mM-Cs^+ to the cell in Fig. 3*B*. In both cells there was block of i_f, more complete in 2 mM-Cs^+ than in 1 mM-Cs^+, but in neither cell was there any increase in the inward current jump at the beginning of the pulse. Indeed,

in the cell in Fig. 3*A*, there was an outward shift in the initial jump and in the holding current indicating that in this cell some i_f was already activated at the holding potential of −40 mV so that block of this caused an outward shift. A similar result (no increase in instantaneous inward current when i_f was blocked by 1 or 2 mM-Cs^+) was found in sixteen out of nineteen cells.

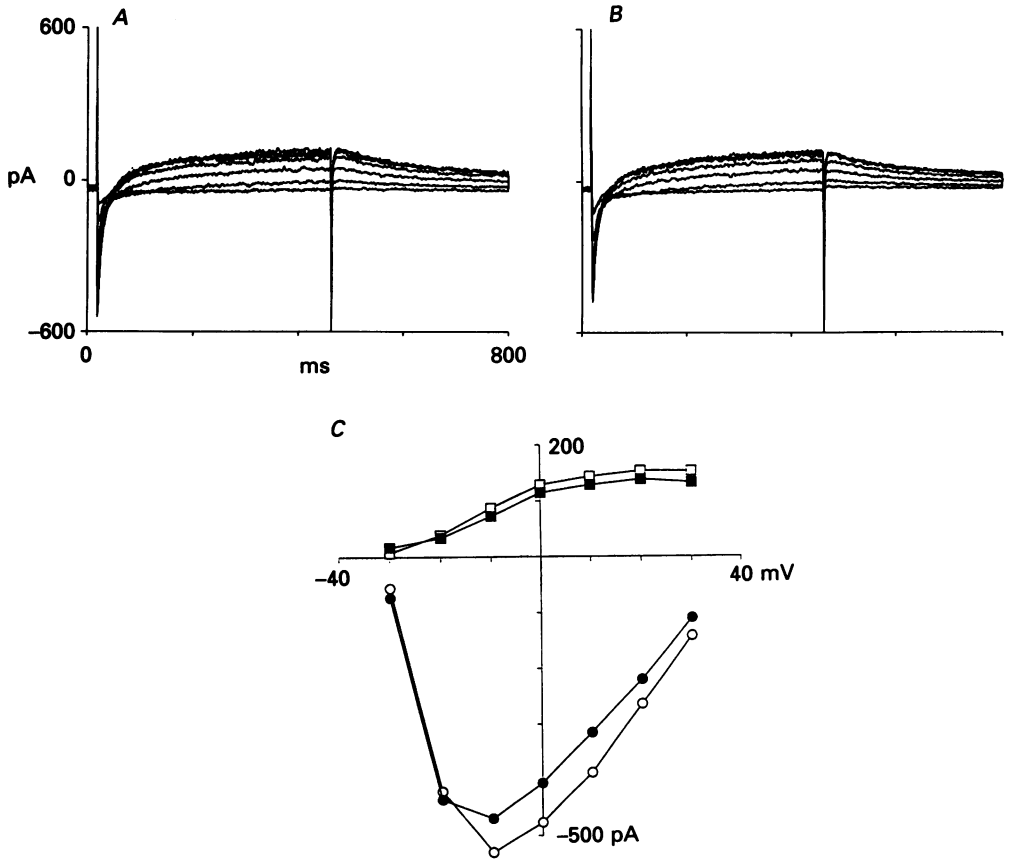

Fig. 4. *A* and *B*, currents recorded using the whole-cell nystatin-permeabilized patch method from an isolated SA node cell in response to depolarizing voltage clamp pulses from a holding potential of −40 mV in 10 mV steps up to +30 mV. Inward calcium current i_{Ca} is seen first during each pulse, succeeded by the development of outward potassium current i_K. *A*, in normal Tyrode solution. *B*, during superfusion with 2 mM-Cs^+ Tyrode solution. *C*, current–voltage plots for the peak i_{Ca} and the i_K tails in *A* and *B* before (open symbols) and after (filled symbols) superfusion with 2 mM-Cs^+.

We also checked whether 2 mM-Cs^+ caused any change in inward calcium current i_{Ca} or in outward potassium current i_K. When the perforated patch technique is used (in which nystatin is included in the pipette solution to create a patch permeable primarily to small cations; Horn & Marty, 1988), it can be seen that there is very little change in i_{Ca} or i_K in 2 mM-Cs^+ (Fig. 4). In whole-cell patch recordings in which the patch was simply ruptured, there was a faster run-down of both i_{Ca} and i_K during

exposure to and recovery from Cs^+ (see Denyer & Brown, 1990, for a discussion of $i_{Ca,L}$ run-down) but in no case (five cells) was there evidence of changes brought about by Cs^+ *per se*.

DISCUSSION

The results above show that 2 mM-Cs^+ applied to isolated sino-atrial node cells blocks the hyperpolarizing-activated current i_f effectively and selectively in the pacemaking range while not altering other membrane currents in such a way as could account for the continued pacemaker activity. This implies therefore that i_f cannot normally be the only inward current contributing to the first (more negative) part of the SA node pacemaker depolarization. Since there is no evidence for any other time-dependent current activated negative to -50 mV, an inward background current must exist in SA nodal cells which supports a mode of pacemaking controlled by the decay of the potassium current i_K over that part of the pacemaker depolarization which is negative to the calcium current threshold.

The nature of such a background current is still not clear. It is possible that a simple sodium 'leak' current exists but it has not yet been unequivocally demonstrated. It is also probable that there are contributions to inward background current from window currents of $i_{Ca,L}$, $i_{Ca,T}$ and i_{Na} (when present in the SA node cell in question). The question of the magnitude of the inward background current i_b has been addressed by Noble *et al.* (1989). In their single SA node cell model, the total background conductance was set in the model at a value which, during hyperpolarizing voltage clamp steps, gave the same instantaneous current shifts preceding the onset of i_f and i_K as seen in the experimental results of DiFrancesco & Noble (1989). This gives a maximum current density for i_b of approximately 3·7 pA/pF during pacing activity.

One problem is, however, that in whole-cell patch clamped cells, a proportion of the instantaneous current shift recorded experimentally could be attributable to current flowing through the seal leak between the cell membrane and the electrode. We had previously found that a greater slowing was produced by 1 mM-Cs^+ in patched cells than in unpatched cells (Brown & Denyer, 1990) which would imply the presence of an inward seal leak current in the patched cells leading to a lesser effect of i_f block on rate. We have now examined the effects of the application of both 1 and 2 mM-Cs^+ to a larger sample of SA node cells and have failed to find consistent differences in the effect of Cs^+ on rate between patched and unpatched cells. A reason for this altered finding could be the improved condition of the cells in our present sample; the effects of 1 mM-Cs^+ suggests that this could be so. In our preliminary study (Brown & Denyer, 1990) we found that 1 mM-Cs^+ ($n = 5$) caused a 40% slowing in unpatched cells compared to 15% in patched cells ($n = 5$), while in our present samples we have found a 21% slowing in unpatched cells ($n = 5$) compared to a 20% slowing in patched cells ($n = 3$). (We did not test 2 mM-Cs^+ previously; in the present study it has given a 30% slowing in unpatched cells ($n = 13$) compared to a 19% slowing in unpatched cells ($n = 3$).) The greater effect of 1 mM-Cs^+ on rate in the earlier sample, coupled with the suggestion of seal leak in those cells, implies cells in poorer condition. The real test is to record the effects of Cs^+ on the same cell

in both the unpatched and the patched conditions, but this we have only once succeeded in doing (when it gave the same percentage slowing). We conclude that it is as well to be aware of possible seal leak: there is still a suggestion that it is present from our results in 2 mM-Cs^+. If it occurs, it will tend to give more depolarized maximum diastolic potentials and faster pacing rates in patched SA node cells.

The observations of beating rate of unpatched cells (where, of course, there is no question of seal leak current or other alterations caused by the application of a patch electrode *per se*) are therefore particularly important. The block of i_f by 2 mM-Cs^+ caused a reduction of the pacemaking rate in every cell tested (average reduction 30%) and there was good recovery on return to normal Tyrode solution. This reduction in frequency is much greater than the slight slowing in 2 mM-Cs^+ (3% in the case illustrated) found by Noma *et al.* (1983) in multicellular preparations of SA node. In multicellular preparations there are possible problems of non-uniformity both of recording and of access of the superfusing solution to every cell, so it seems justifiable to give weight to the present consistent results from a number of isolated cells.

One of the roles of i_f in SA node is as a modulatory current in the control of pacemaker rate; it has been shown to be enhanced by β-adrenergic agents (Brown, DiFrancesco & Noble, 1979; DiFrancesco, Ferroni, Mazzanti & Tromba, 1986; Hagiwara & Irisawa, 1989) and to be reduced by acetylcholine (DiFrancesco, DuCouret & Robinson, 1989). The demonstration that blocking i_f causes a marked effect on pacemaker rate in every isolated SA node cell tested confirms i_f in its role as an important modulator of heart rate.

We should like to thank Professor Denis Noble FRS and Dr Trevor Powell for discussion and encouragement and Michael Kilborn and Tony Spindler for their help. The work was supported by the British Heart Foundation, the Wellcome Trust, the MRC and the SERC.

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