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PACEMAKER CURRENT IN SINGLE CELLS AND IN AGGREGATES OF CELLS DISSOCIATED FROM THE EMBRYONIC CHICK HEART

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SUMMARY

1. We have measured the time-dependent pacemaker current, I_f, in single cells, or small clusters of two or three cells dissociated from embryonic chick hearts with the whole-cell patch clamp technique, and in multicellular reaggregates of dissociated cells with the two-microelectrode voltage clamp technique.

2. We observed time-dependent current (I_f) in the -90 to -60 mV range from aggregates of ventricular cells, as in our earlier results from this preparation, which we previously attributed to the potassium ion current mechanism, I_{K_2}. We also observed I_f in single atrial cells and aggregates of atrial cells.

3. The range of activation of I_f was -120 to -90 mV in atrial preparations (either single cells or aggregates). The activation range of I_f in ventricular cells was also -120 to -90 mV which is ~ 30 mV negative to the I_f activation range in ventricular cell aggregates. The reasons for this shift of I_f in ventricular preparations are unknown.

4. The I_f component clearly underlies the spontaneous pacemaker depolarization which is observed in ventricular heart cell aggregates during the first week of embryonic development. However, I_f is not a significant factor underlying spontaneous activity in atrial preparations. The pacemaker current in these cells is a net inward background component, which is significantly reduced in amplitude with development, as is the I_f component in the ventricle.

INTRODUCTION

The pacemaker current in the mammalian sino-atrial node is the I_f component, a membrane ion conductance which is activated with membrane hyperpolarization, and which passes current in the net inward direction for pacemaker potentials

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(DiFrancesco, 1985). This conductance is also present in cardiac Purkinje fibres, in which it was originally misinterpreted as an outward, potassium ion component, I_{K_2} (Noble & Tsien, 1968; DiFrancesco, 1981*a*, *b*). Several years ago we reported a pacemaker current in aggregates of embryonic chick ventricular heart cells, which we attributed to I_{K_2} (Shrier & Clay, 1980; Clay & Shrier, 1981*a*, *b*). We have reinvestigated this component in aggregates of ventricular and atrial heart cells from 4- to 14-day-old chick hearts using the two-microelectrode voltage clamp technique, and in single cells from both the ventricle and the atrium using the patch clamp technique. We have concluded that the pacemaker current in our preparations is indeed I_f. The misinterpretation of I_f as I_{K_2} was due, in our view, to a mixture of inwardly directed I_f activation kinetics and outwardly directed inactivation kinetics of the background potassium ion current, I_{K_1}, which can lead to the I_{K_2} interpretation, if both time-dependent features are attributed to the same ion conductance mechanism.

A surprising result of this analysis is that the position of the I_f activation curve on the voltage axis in single ventricular cells, or small clusters of two to three cells, is approximately 30 mV negative (−90 to −120 mV) to the activation range of I_f in aggregates of cells (−60 to −90 mV). This voltage shift does not appear to be attributable to a difference between multicellular and single-cell preparations *per se*, because I_f in embryonic chick atrial cell preparations has the same range of activation (−90 to −120 mV) in single cells as in aggregates. Moreover, the voltage shift of the I_f activation curve in the ventricle does not appear to be caused by a difference in recording technique, because a shift in the I_f activation curve in single ventricular cells relative to the aggregate results was also observed with the perforated patch clamp technique.

The I_f component is clearly involved in generating spontaneous pacemaker depolarization in aggregates of embryonic ventricular cells. A time-independent (background) component in the net inward direction underlies spontaneous activity in atrial cell preparations (Shrier & Clay, 1986). Both the I_f and the background components are significantly reduced with embryonic development, as we previously demonstrated (Shrier & Clay, 1980; Clay & Shrier, 1981*b*).

A brief report of some of these results has appeared (Brochu, Clay & Shrier, 1990).

METHODS

Tissue culture techniques

This paper contains electrophysiological results from cellular aggregates, or more correctly, reaggregates containing several hundred cells (single-cell diameter ~15 μm; aggregate diameter ~100–200 μm) and isolated single cells or small cellular clusters containing two to three cells. The dissociation procedures used for both types of preparations were the same as described in Shrier & Clay (1986). Either the atria or the ventricles were dissected from the hearts of White Leghorn chicken embryos (*Gallus gallus*) incubated at 37 °C for 4–14 days. The tissue was enzymatically dispersed in 0·5% trypsin (1:300, Worthington Biochemical Co., Freehold, NJ, USA) by the multiple-cycle trypsinization process described by DeHaan (1967, 1970). These procedures yield a suspension consisting primarily of isolated, single cells. An inoculum of this suspension was added to a plastic tissue culture dish containing culture medium 818A (DeHaan, 1970) which consists, by volume, of 25% M199 (Gibco, Grand Island, NY, USA), 2% heat-inactivated, selected horse serum (Gibco), 4% heat-inactivated fetal calf serum (Gibco), and 0·5% gentamycin (Schering Corp., Bloomfield, NY, USA) in a balanced salt solution which contained (in mM): NaCl, 116·0;

$MgCl_2$, 0·8; NaH_2PO_4, 0·9; $CaCl_2$, 1·8; $NaHCO_3$, 26·6; glucose, 5·5; KCl, 1·3. The cells were placed in a tissue culture dish in an incubator for 1–5 days at 37 °C in an atmosphere consisting of 5 % CO_2, 10 % O_2 and 85 % N_2. Typically, the cells remained isolated from one another as they migrated to the bottom of the dish. Occasionally, small clusters were formed which contained a few cells, at most. Cellular aggregates were prepared by adding an inoculum of cells to 3 ml of medium 818A which was then added to an Erlenmeyer flask. The flask was gassed with 5 % CO_2, 10 % O_2, and 85 % N_2, sealed with a silicone rubber stopper, and placed on a gyratory shaker for 1–3 days at 37 °C. During this time, spherical aggregates were formed having diameters typically in the 100–300 μm range (Sachs & DeHaan, 1973). After the gyration culture procedure, the contents of the flask were transferred to a plastic tissue culture dish. The aggregates migrated to the bottom of the dish to which they firmly adhered.

Electrophysiology

The whole-cell configuration of the patch clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) was used for voltage clamping single dissociated cells, or small clusters of two or three cells at room temperature (T = 24 °C). Patch clamp electrodes were filled with 'intracellular' medium containing (in mM): KCl, 140; NaCl, 5; $MgSO_4.7H_2O$, 1; EGTA, 1; glucose, 5; ATP, 1; HEPES buffer (*N*-2-hyroxyethylpiperazine-*N*-2-ethansulphonic acid), 10; pH = 7·4. The external medium contained (in mM): NaCl, 140; $MgCl_2$, 1; NaH_2PO_4, 0·2; $CaCl_2$, 1·8; HEPES, 10; glucose, 5; and KCl, as indicated in the text by the value given for the external potassium ion concentration, K_o^+. Experiments with barium were carried out by adding $BaCl_2$ to the external medium at a final concentration of 1–5 mM. In most experiments tetrodotoxin (TTX; 0·5 μM) was used to block the I_{Na} component. The measurements of the I_f reversal potential (Fig.5) and I_f tail current kinetics for $V > -60$ mV were carried out with 2 mM-$MnCl_2$ to block the I_{Ca} component. The liquid junction potential between the intracellular medium inside the patch clamp electrode and the extracellular medium was compensated. A List EPC-7 amplifier was used for recording membrane currents (List Medical Equipment, Darmstadt, FRG). The establishment of whole-cell recording was confirmed by monitoring the increase in capacitance as the patch was broken, and by monitoring membrane potential in current-clamp mode after patch disruption. Total series resistance (including electrode resistance) was typically 3–5 MΩ, of which more than 50 % could be compensated.

Experiments using the perforated patch clamp technique were carried out using techniques described by Horn & Marty (1988). Nystatin (Sigma, St Louis, MO, USA) was freshly dissolved in dimethylsulphoxide (DMSO; 5 mg ml^{-1}) and subsequently added to the 'intracellular' (internal) solution with the aid of a sonicator at a final concentration of 50–100 μg ml^{-1}. This solution was used for up to 2 h. The tip of the patch clamp electrode was filled with normal internal medium. The rest of the electrode was back-filled with the nystatin-containing solution. A gigaseal on a cell in the tissue culture dish was rapidly formed, usually within 2–4 min. The electrode potential was subsequently observed to increase over the course of several minutes and reach a steady level (−25 to −35 mV in the presence of 1 mM-$BaCl_2$). Voltage clamp recordings were then carried out usually with a holding potential of −60 mV.

Voltage clamp measurements from aggregates were carried out at 37 °C with two glass microelectrodes containing 3 M-KCl which were impaled within two different cells of an aggregate. The electrodes were connected to a standard voltage clamp circuit. The recordings of spontaneous activity in Fig. 8*A* and *D* were obtained with a single electrode impaled within one of the cells in the aggregate in medium 818A with K_o^+ = 1·3 mM and without TTX. The suitability of heart cell aggregates for the voltage clamp technique is discussed in Clay, DeFelice & DeHaan (1979).

Data recording and analysis

Membrane currents in the single-cell work were filtered at 10 kHz and recorded on a video cassette recorder by means of a pulse code modulation (PCM) digitizing unit (Sony Corp., New York, NY, USA). These records were filtered during off-line analysis (low pass, 3 dB cut-off at 200 Hz) and digitized at 10 ms intervals. The bulk of our membrane current kinetics in the presence of barium could be described by a single-exponential process, which was routinely used to fit our results. Membrane currents in the work on aggregates were recorded on a Hewlett-Packard tape-recorder (3964A) at $3\frac{3}{4}$ in s^{-1} (DC-1250 Hz). These results were filtered during off-line analysis (low pass; 3 dB cut-off at 200 Hz).

RESULTS

Control results from ventricular cells

Representative pacemaker currents in this study from an aggregate are illustrated by the results in Fig. 1*A* (5-day old cells; aggregate diameter = 150 μm; K_o^+ = 4 mM;

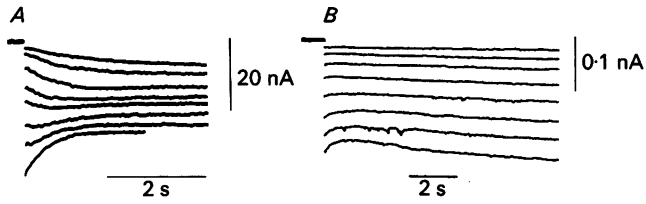

Fig. 1. *A*, membrane current records from a 150 μm diameter aggregate of 5-day-old embryonic chick ventricular heart cells. Step potentials: −82, −88, −94, −102, −105, −109, −112, −117 mV. Holding potential; −67 mV. K_o^+ = 4 mM. T = 37 °C. *B*, membrane current records from a two-cell cluster of 7-day-old ventricular cells. Step potentials: −70, −80, ..., −140 mV. Holding potential: −60 mV. K_o^+ = 6 mM. T = 24 °C.

holding potential = −67 mV; step potentials indicated in the legend of Fig. 1). The membrane current records for the steps to −82 and −88 mV are consistent with our earlier results from aggregates of 7-day-old ventricular heart cells (Clay & Shrier, 1981*a*). In particular, a clear, inwardly directed time dependence is evident in these results having a time constant $\lesssim$ 1 s (T = 37 °C), which is voltage dependent. The record for −117 mV illustrates an outwardly directed time dependence having a relatively slow time course, which is also similar to our earlier results (Fig. 6 of Clay & Shrier, 1981*a*). The records in Fig. 1*A* for intermediate potentials, in particular V = −94, −102 and −105 mV, illustrate a biphasic time dependence, a kinetic feature that we did not observe previously. By contrast, the records in Fig. 1*B* from a two-cell cluster (7-day-old cells; K_o^+ = 6 mM) do not show a clear time-dependent pacemaker component in the −90 to −60 mV membrane potential range. This feature is evident at more negative potentials, especially −130 and −140 mV, having a time constant > 1 s (T = 24 °C) along with an outwardly directed time dependence. Consequently, these records have a biphasic appearance. Results similar to those in Fig. 1*B* were observed in nine out of fifteen single-cell or small cell-cluster preparations. In the other six preparations we observed only the outwardly directed component.

Effects of potassium and barium

The effect of a change in the external potassium ion concentration, K_o^+, on I_f is illustrated in Fig. 2*A* and *B* (7-day-old cells). The results in Fig. 2*A* with 1 mM-K_o^+ clearly show an I_f component, especially for steps to −110 and −120 mV. An increase of K_o^+ to 5 mM revealed a significant increase in the amplitude of current jumps in the inward direction following hyperpolarizing voltage steps. These results also illustrate an outwardly directed time dependence, and surprisingly, an apparent *reduction* in the I_f amplitude. The I_f component was once again evident following the addition of 5 mM-$BaCl_2$ to the external medium (Fig. 2*C*). A significant increase in I_f

amplitude was observed following an increase in K_o^+ to 50 mM, which is similar to the effect of high levels of K_o^+ on I_f in cardiac Purkinje fibres (DiFrancesco, 1981*b*). The effects of Ba^{2+} on the aggregate preparation are illustrated in Fig. 3 (7-day-old cells; 100 μm diameter aggregate; $K_o^+ = 4$ mM). These results are strikingly similar, at

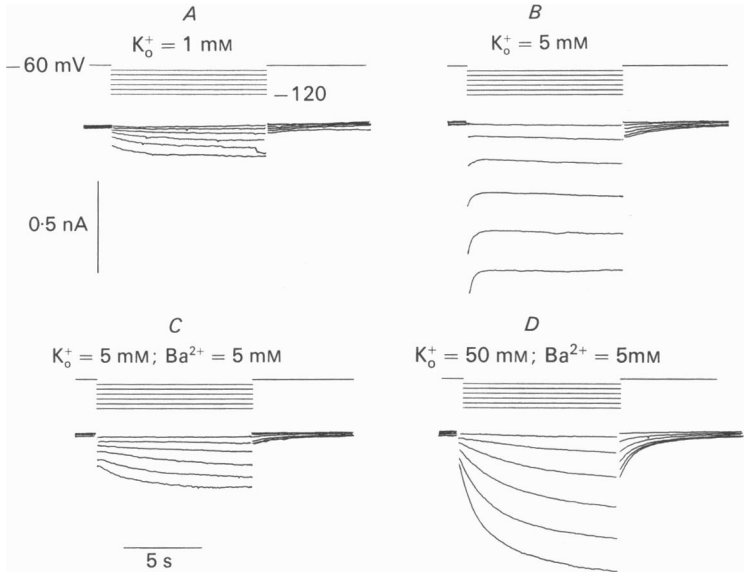

Fig. 2. *A*, membrane current records from a three-cell cluster of 7-day-old embryonic chick ventricular heart cells. $K_o^+ = 1$ mM. *B*, same preparation as in *A* with $K_o^+ = 5$ mM. *C*, effect of 5 mM-Ba^{2+}. *D*, increase in I_f with 50 mM-K_o^+.

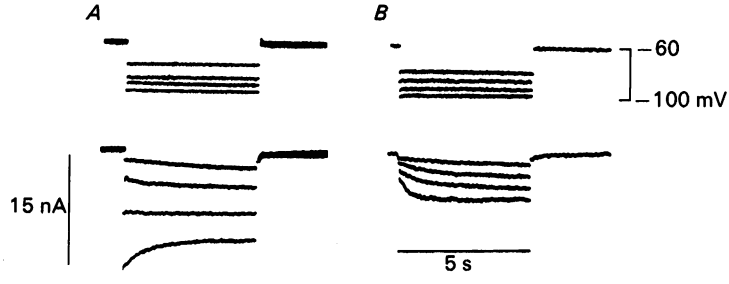

Fig. 3. *A*, membrane current records from a 100 μm diameter aggregate of 7-day-old embryonic chick ventricular heart cells. Step potentials: −78, −87, −95, and −102 mV. Holding potential: −60 mV. *B*, effects of 5 mM-Ba^{2+}. Same preparation as in *A*. Step potentials: −80, −88, −95, −100 mV. $K_o^+ = 4$ mM. $T = 37$ °C.

least qualitatively, to the results from the small cluster in Fig. 2*B* and *C*. Similar results were obtained with barium (either 1 or 5 mM) in a total of five aggregates.

Comparison of I_f kinetics from single cells and aggregates

The qualitative impression above that I_f is activated at relatively more negative potentials in single ventricular cells, or small clusters of ventricular cells, as

compared to ventricular cell aggregates is further illustrated in Fig. 4. The experimental results in Fig. 4*A* describe the relative voltage dependence of the I_f deactivation, or 'tail' current, either in control or with Ba^{2+}, upon stepping back to the holding potential from five different single-cell or small cell-cluster preparations

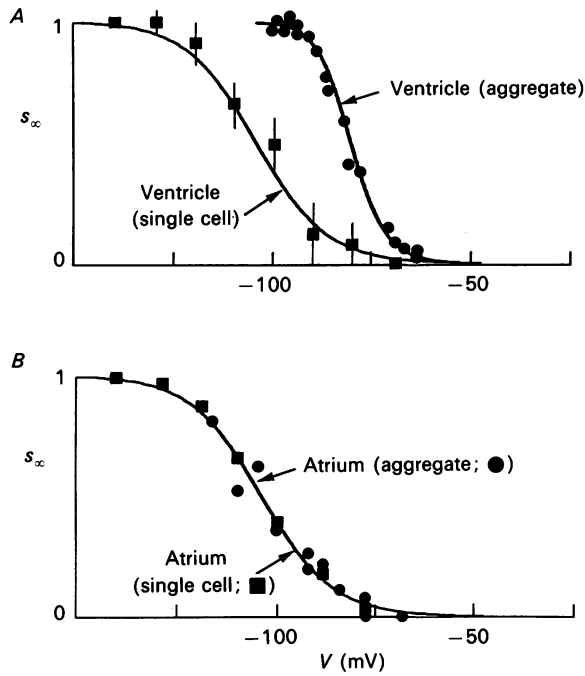

Fig. 4. *A*, normalized tail current amplitudes of I_f from single ventricular cells or small clusters of ventricular cells (■; $n = 5$; ±S.D.), and from an aggregate of ventricular cells (●). The latter results were re-plotted from Fig. 5*B* of Clay & Shrier (1981*a*) according to the I_f rather than the I_{K_2} mechanism, as described in the text. The single-cell results were described by the theoretical relation $s_\infty = \beta_s/(\alpha_s+\beta_s)$ with $\alpha_s = 0{\cdot}17(V+48)/(1-\exp(-0{\cdot}07(V+48)))$ s^{-1} and $\beta_s = 0{\cdot}0052\exp(-0{\cdot}06(V+48))$ s^{-1} with V in mV. The aggregate results were described with $\alpha_s = 1{\cdot}05(V+62)/(1-\exp(-0{\cdot}2(V+62)))$ s^{-1} and $\beta_s = 0{\cdot}095\exp(-0{\cdot}075(V+62))$ s^{-1}, as in Clay & Shrier (1981*a*), but with the roles of α_s and β_s reversed. $K_o^+ = 1$–6 mM in the single-cell work; 1·2–4·8 mM in the aggregate work. *B*, normalized tail current amplitudes of I_f from a two-cell cluster of atrial cells (■), and from two different aggregates of atrial cells (●). The continuous line is the same as the one used to describe the single ventricular cell results in *A*.

(denoted by the symbol ■). For comparison, similar results are also shown from an aggregate of ventricular heart cells from Clay & Shrier (1981*a*; denoted by the symbol ● in Fig. 4*A*) with the tail current amplitudes plotted according to the I_f interpretation rather than the I_{K_2} interpretation. (The pacemaker current kinetics are the same in both interpretations. The only modification is that the tail current amplitudes are inverted around the midpoint of the activation curve.) As we noted previously (Clay & Shrier, 1981*a*), the midpoint of the activation range of the pacemaker current in aggregates was $-80{\cdot}0 \pm 3{\cdot}4$ mV ($n = 15$; ±S.D.). The midpoint of activation of I_f in single ventricular cells or small clusters of cells was $-102{\cdot}6 \pm 5{\cdot}4$ mV ($n = 5$; ±S.D.). The continuous line in the left-hand part of Fig. 4*A*

is a description of the single-cell and small cluster results using the expression $s_\infty = \beta_s/(\alpha_s+\beta_s)$, where α_s and β_s are given in the legend of Fig. 4. The continuous line in the right-hand part of Fig. 4*A* is a description of tail currents from aggregates taken from Clay & Shrier (1981*a*) with α_s and β_s as given in our previous work, but with the roles of α_s and β_s reversed. The results in Fig. 4*A* demonstrate that the

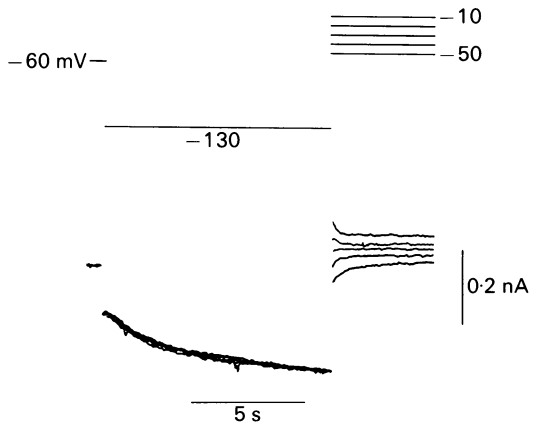

Fig. 5. Reversal potential of I_f. Single cell; 14 days old; $K_o^+ = 10$ mM; $Ba^{2+} = 5$ mM; $Mg^{2+} = 2$ mM. Voltage clamp protocol shown above the current records.

midpoint of the activation range of I_f in single cells is shifted negative to the corresponding result in aggregates. The amplitude of the shift is $22{\cdot}6 \pm 6{\cdot}3$ mV. Moreover, the activation curve is less steeply voltage dependent in single cells, as compared to aggregates.

Reversal potential

The results in Fig. 1*A*, if attributed to a single kinetic component, are suggestive of a reversal potential close to the potassium ion equilibrium potential, E_K. However, the experiments with barium demonstrate that the inwardly directed component in these records cannot be a potassium ion selective current. The reversal potential, E_f, is positive to the range of activation of the pacemaker kinetics, as demonstrated in cardiac Purkinje fibres by DiFrancesco (1981*a*). We have obtained similar results as illustrated in Fig. 5 (single cell, 14 days old, $K_o^+ = 10$ mM, $Ba^{2+} = 5$ mM), which give $E_f \sim -30$ mV. The range of our measurements of E_f from single cells and small clusters of cells was -30 to -10 mV ($n = 5$). We did not systematically investigate the dependence of this result on ionic conditions. DiFrancesco (1981*b*) has shown that the I_f component is permeable both to sodium and potassium ions.

Pacemaker current in atrial cells

We previously reported the absence of a time-dependent pacemaker current both in aggregates of atrial cells and in single, isolated cells in the -90 to -60 mV range (Shrier & Clay, 1986; Clay, Hill, Roitman & Shrier, 1988). This result is further illustrated in Fig. 6, which contains results from a 200 μm diameter aggregate of 7-

day-old atrial cells and a two-cell cluster of 7-day-old atrial cells, Fig. 6*A* and *B* respectively. These experiments were extended to potentials more negative than −90 mV, where an I_f component was evident. Moreover, the range of activation of this current was the same in single cells or small clusters of cells as in aggregates, as

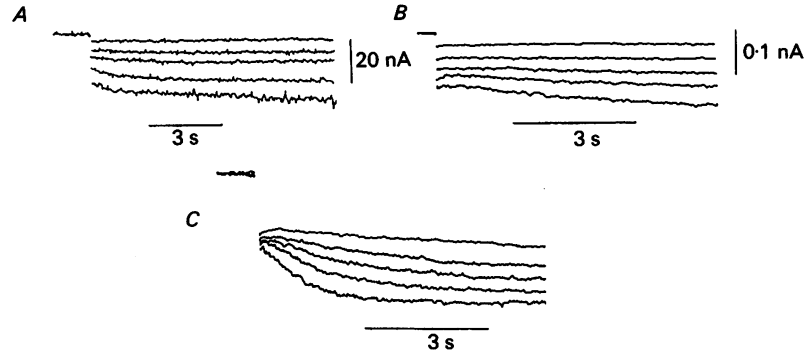

Fig. 6. *A*, membrane currents from a 200 μm diameter aggregate of 7-day-old atrial cells. Step potentials; −70, −80, ..., −110 mV. Holding potential: −60 mV. K_o^+ = 1·3 mM. T = 37 °C. *B*, membrane currents from a two-cell cluster of 7-day-old atrial cells. Step potentials; −70, −80, ..., −110 mV. Holding potential: −60 mV. K_o^+ = 1·3 mM. T = 24 °C. *C*, same preparation as in *B*. Holding potential = −25 mV. Step potentials = −100, −110, ..., −140 mV.

illustrated in Fig. 4*B*. We also note that the relatively negative range of activation of I_f in atrial preparations is similar to the activation of I_f in single ventricular cells shown in Fig. 4*A*. The I_f component from the preparation described in Fig. 6*B* is illustrated for larger steps in Fig. 6*C* (holding potential −25 mV). Results similar to those in Fig. 6 were observed in six atrial aggregates and in three single atrial cells, or small clusters of atrial cells. The I_f component was not present in four other single atrial cells. These I_f results, which have not been previously reported from embryonic chick atrial heart cells, are similar to the measurements of I_f in sheep atrium by Earm, Shimoni & Spindler, (1983). The time-independent, net inward background current (the instantaneous current jumps in Fig. 6, especially *A* and *B*) was evident in all preparations. This background component appears to be the pacemaker current underlying spontaneous activity in atrial cells (Shrier & Clay, 1986).

Results with the perforated patch technique

Experiments were carried out on single ventricular cells with nystatin-containing pipettes (Methods) to test the hypothesis that the differences in the aggregate and single-cell results concerning the I_f activation curve (Fig. 4) might be attributable to washout of an intracellular factor during suction pipette recording. Voltage clamp results obtained with this technique are illustrated in Fig. 7. Voltage steps to −90 mV, or less negative levels, did not elicit a time-dependent component. A clear time-dependent current was evident with steps to −110 and −120 mV, as shown in Fig. 7. Similar results were obtained in three other preparations. That is, the activation range of the I_f component in single ventricular cells as measured with the

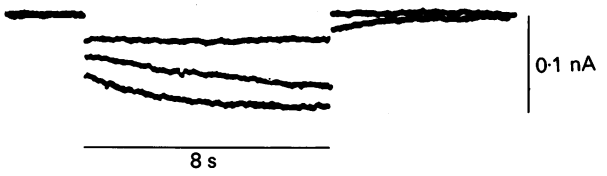

Fig. 7. I_f as measured with the perforated patch technique (Methods) from a 5-day-old ventricular cell. $K_o^+ = 5$ mM; $Ba^{2+} = 1$ mM. $T = 24$ °C. Steps to −90, −110 and −120 mV. The tail currents for −90 and −120 mV only are shown.

perforated patch clamp technique appeared to be similar to the results obtained with the suction pipette technique. Moreover, the activation range of the delayed rectifier component, I_K, was not significantly different from our earlier measurements of I_K obtained with the suction pipette technique (Clay *et al.* 1988).

DISCUSSION

We have reinvestigated the pacemaker current component in embryonic chick heart cells using single cells, small clusters of cells, and reaggregates containing several hundred cells. The advantage of this approach is that it permits a comparison of measurements of an ionic current component from single cells with similar measurements from multicellular preparations under conditions in which the single cell and the multicellular preparations were obtained from the heart with the same tissue culture techniques. We recently demonstrated with this approach that the kinetics of the delayed rectifier component, I_K, were the same in single cells as in aggregates (Clay *et al.* 1988). We obtained a similar result in this study concerning I_f in atrial cells. The comparison concerning I_f in ventricular preparations revealed a different result, namely, a shift of the I_f activation curve along the voltage axis in the negative direction in single cells relative to aggregates. One possible explanation for this result might be a difference in recording technique. The results from aggregates were obtained with intracellular electrodes which were impaled in cells within the aggregate, whereas the results from single cells in Fig. 4*A* were obtained with the suction pipette technique, which might have 'washed out' an intracellular factor that regulates I_f in the ventricle. The results in Fig. 7 with the perforated patch technique argue against this idea. Rather, the shift appears to be attributable to an intrinsic difference in the I_f component in single, isolated cells, or small clusters of cells, compared to the I_f component in cells which are within aggregates containing a few hundred cells.

Clearly, the ionic mechanism underlying the inwardly directed time-dependent current for voltages negative to −60 mV in both the multicellular and single cell preparations in this analysis is, indeed, attributable to I_f rather than I_{K_2}. The evidence for this re-interpretation is similar to that given by DiFrancesco (1981*a*), in particular the effects of Ba^{2+} in Figs 2*B*, *C* and 3, which demonstrate that the pacemaker current cannot be attributable to a potassium ion-selective conductance. The qualitative similarity of the effect of Ba^{2+} on pacemaker currents in aggregates and in small clusters (Figs 2*B*, *C* and 3) together with the results in Fig. 2*A* and *B*

concerning the effects of K_o^+ on pacemaker current waveforms in control conditions provide additional insight to this issue. The potassium ion equilibrium potential, E_K, with $K_o^+ = 1$ mM is ~ -125 mV, which is negative to the range of measurements in Fig. 2*A* (-120 to -60 mV). Consequently, the background potassium ion current, I_{K_1} does not contribute, significantly, to these waveforms because of its marked inward rectification for $V > E_K$ (Sakmann & Trube, 1984*a*). An increase of K_o^+ from 1 to 5 mM shifts the I_{K_1} current–voltage relation along the voltage axis so that the inward current portion of this relation is within the voltage range of measurements in Fig. 2*A* and *B*. Consequently, the marked increase in the inward current jumps in Fig. 2*B* is clearly attributable to I_{K_1}. Moreover, the outwardly directed time dependence in these records can be attributed to the time-dependent inactivation of I_{K_1} (Sakmann & Trube, 1984*b*). A surprising feature of these results is the apparent diminution of the I_f amplitude, especially evident at -110 and -120 mV with $K_o^+ = 5$ mM. This effect appears to be attributable to a slow phase of I_{K_1} inactivation which masks I_f, based upon the results with barium in Fig. 2*C*, and which is known to block I_{K_1} (Sakmann & Trube, 1984*b*). These results suggest that the apparent reversal of pacemaker current in control conditions (the 'I_{K_2} reversal'), which was the primary basis for the previous interpretation of pacemaker current both in our preparations as well as in cardiac Purkinje fibres as I_{K_2} (Noble & Tsien, 1968), is attributable to the time-dependent inactivation of I_{K_1}. DiFrancesco & Noble (1985) attributed the 'I_{K_2} reversal' to the effect of potassium ion depletion in the extracellular spaces of multicellular preparations on a time-independent I_{K_1} component during a hyperpolarizing voltage clamp step. This model predicts monophasic pacemaker currents (the I_f component alone) in single cells or small clusters of cells, which is inconsistent with our results and also with results from single cardiac Purkinje fibre cells (Callewaert, Carmeliet & Verecke, 1984). The latter group has also emphasized the contribution of I_{K_1} time dependence to pacemaker current waveforms. This mechanism offers an alternative explanation for the marked effect of Ba^{2+} on pacemaker currents in multicellular preparations, such as heart cell aggregates and canine cardiac Purkinje fibres, in which ion accumulation and depletion are believed to be considerably less pronounced than in sheep cardiac Purkinje fibres (Clay *et al.* 1979; Cohen, Falk & Mulrine, 1983).

The mechanisms which underlie spontaneous activity in embryonic chick heart cells can be elucidated by incorporating the results in this study into our model of electrical activity in atrial heart cell aggregates (Shrier & Clay, 1986). We have previously shown that the net inward (background) component is sufficient to describe the spontaneous pacemaker depolarization in these preparations in control conditions. Moreover, we have recently shown that the model is sufficient to describe, in considerable detail, the influences on this activity of brief duration current pulses (Clay, Brochu & Shrier, 1990). We have demonstrated here that atrial preparations also possess an I_f component, activated at relatively negative potentials, which we did not include in our earlier simulations. We have assessed the contribution of this current to electrical activity in atrial aggregates by including a theoretical description of the I_f results given in Figs 4 and 6 in our model. We have used $I_f = g_f s(V,t)(V-E_f)$, where $g_f = 1$ mS cm^{-2}, $E_f = -30$ mV, and $s(V,t) = -s(V,t)/\tau_f + \beta_f$, where $\tau_f = (\alpha_f + \beta_f)^{-1}$, and α_f and β_f are as given in the legend of Fig. 4. An example of electrical

activity from a spontaneously beating aggregate of atrial heart cells (7 days old) is shown in Fig. 8*A*. The prediction of our model with I_f included is shown in Fig. 8*B* with the background current adjusted so as to closely match the frequency of activity of the experimental result. The prediction of the model without I_f included is shown in

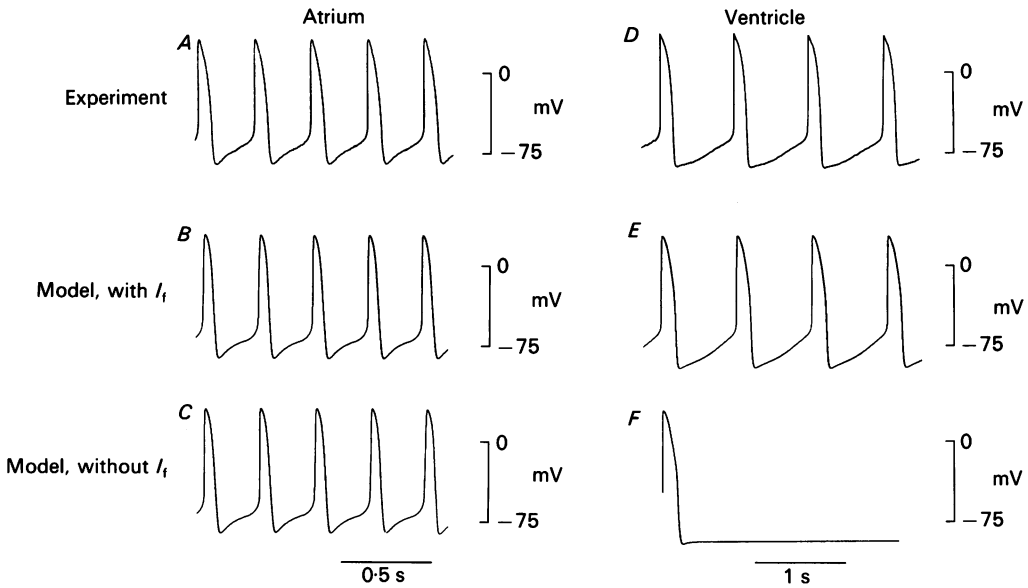

Fig. 8. *A*, spontaneous electrical activity from an aggregate of atrial cells (7 days old). $K_o^+ = 1{\cdot}3$ mM. $T = 37$ °C. *B*, prediction from the Shrier & Clay (1986) model of electrical activity from atrial cell aggregates with $I_{b_1} = 0{\cdot}07(V+40)$ rather than $0{\cdot}042(V+40)$ and with the fully activated current–voltage relation for I_{x_1} replaced by $30(V+98)/(1+4\exp(0{\cdot}75(V+98)))$. Also, the I_f component is included in this simulation, as described in the text, where $I_f = g_f s(V,t)(V,E_f)$, where $g_f = 0{\cdot}023$; $E_f = -30$ mV; and $ds(V,t)/dt = -s/\tau_s + \beta_s$, where $\alpha_s = 1{\cdot}56(V+67)/(1-\exp(-0{\cdot}07(V+67)))$ and $\beta_s = 0{\cdot}052\exp(-0{\cdot}06(V+67))$. *C*, same as *B* without the I_f component. *D*, spontaneous electrical activity from an aggregate of ventricular heart cells (7 days old). $K_o^+ = 1{\cdot}3$ mM. $T = 37$ °C. *E*, prediction of the Shrier & Clay (1986) model with I_{x_1} modified as described in *B* and with the background current ($I_{b_1}+I_{b_2}+I_{b_3}$) replaced by $2{\cdot}8(V+95)/(1+4\exp(3{\cdot}2(V+95)))$. The I_f component was also included in this simulation as described in *B* but with α_s and β_s as given in Clay & Shrier (1981*a*). That is, $\alpha_s = 1{\cdot}05(V+62)/(1-\exp(-0{\cdot}2(V+62)))$ and $\beta_s = 0{\cdot}095\exp(0{\cdot}075(V+62))$. *F*, same as *E* but with I_f removed.

Fig. 8*C*. The results in Fig. 8*B* and *C* differ in frequency by only 2%. That is, the addition of I_f to the model does not make a clear difference in the theoretical result, because the activation range of I_f in atrial cells lies below the physiological range of membrane potentials. The I_f component clearly does contribute to electrical activity in aggregates of ventricular cells, as illustrated in Fig. 8*D–F*. The experimental result in Fig. 8*D* was taken from an aggregate of 7-day-old ventricular cells which had a significant I_f component based on subsequent voltage clamp analysis of this preparation. The theoretical result in Fig. 8*E* was obtained from our atrial cell model with the background current modified so that this component was in the net outward direction for pacemaker potentials (Fig. 2 of Clay & Shrier, 1981*b*) and with I_f as

described above but with α_f and β_f as given in Clay & Shrier (1981*a*). The prediction of the model without I_f is shown in Fig. 8*F*. The model does not produce spontaneous activity without I_f, in contrast to the atrial cell result in Fig. 8*C*.

In conclusion we note that spontaneous activity is not as apparent in either atrial or ventricular chick heart cell preparations obtained from embryos just prior to hatching as it is in cells derived from younger embryos (Shrier & Clay, 1980; Clay & Shrier, 1981*b*; and authors' unpublished observations). The reasons for these developmental changes appear to be a developmental loss of I_f in the ventricle and a developmental loss of the inward background component in the atrium.

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