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Comparison of the Pacemaker Properties of Chick Embryonic Atrial and Ventricular Heart Cells

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Summary. We have investigated the pacemaker properties of aggregates of cells dissociated from the atria and ventricles of 10 to 14-day-old chick embryonic hearts using a two-microelectrode current and voltage-clamp technique. These preparations usually beat spontaneously and rhythmically in tissue culture medium containing 1.3 mM potassium with a beat rate typically in the range of 15–60 beats per minute. The beat rate results show considerable variability, which precludes any statistically significant comparison between the spontaneous activity of atrial and ventricular cell preparations at 10–14 days of development. However, the shapes of pacemaker voltage changes do exhibit differences characteristic of cell type. Spontaneous atrial preparations rapidly depolarize from maximum diastolic potential (~ -90 mV) to a plateau range of pacemaker potentials (-80 to -75 mV). The membrane subsequently depolarizes more gradually until threshold (~ -65 mV) is reached. In contrast, spontaneously beating ventricular cell preparations slowly hyperpolarize after maximum diastolic potential to the -100 to -95 mV range before gradually depolarizing toward threshold. Voltage-clamp analysis reveals a virtual lack of any time-dependent pacemaker current in atrial preparations. These preparations are characterized by an approximately linear background current (I_{bg}) having a slope resistance of $\sim 100\ \mathrm{K\Omega\,cm^2}$. Ventricular preparations have a potassium ion pacemaker current with slow kinetics (I_{K_2}), and a second time-dependent component (I_x) which is activated at potentials positive to -65 mV. The background current of these preparations displays inward rectification. Computer simulations of pacemaking reveal that the initial rapid phase of pacemaker depolarization in atrial cells is determined by the membrane time constant, which is the product of membrane capacitance and the slope resistance of I_{bg}. The hyperpolarization after maximum diastolic potential of ventricular cells is caused by I_{K_2}. The final slow phase of depolarization in both cell types is caused in part by the steady-state amplitude of the fast inward sodium current (I_{Na}). This component has negative slope conductance which effectively increases the slope resistance in the vicinity of threshold compared to TTX-treated preparations. This mechanism is sufficient to produce interbeat intervals several seconds in duration, even in the absence of time-dependent pacemaker current, provided that the background current is at the appropriate level.

Key words embryonic heart · tissue culture · pacemaking mechanisms · voltage-clamp

Introduction

Individual cells and reaggregates of cells dissociated from the hearts of chick embryos beat spontaneously and rhythmically at a rate which usually reflects the region of the heart from which the cells were derived (Cavanaugh, 1955; DeHaan, 1970; Sachs & DeHaan, 1973). Specifically, atrial cells beat faster than do cells isolated from ventricle during the first week of development which is consistent with observations on the beat rate of fragments of atrial and ventricular tissue dissected from the intact embryonic heart (Barry, 1942; Cavanaugh, 1955; Sachs & DeHaan, 1973). However, beat rate studies on isolated cells and aggregates show considerable variability. For aggregates the variability is due in part to a dependence of rate upon the number of cells within a given preparation; aggregates consisting of more than a few hundred cells beat at a rate which is inversely proportional to cell number, whereas smaller preparations do not show a size-rate dependence (Sachs & DeHaan, 1973; Clay & DeHaan, 1979). A further source of variability lies in the dependence of spontaneous activity on development, since both atrial and ventricle cells lose their automaticity during the incubation period (DeHaan, 1970; Sachs & DeHaan, 1973; Shrier & Clay, 1980; Clay & Shrier, 1981*b*). However, beat rate results show considerable variability within a group of preparations even when size, developmental age, and tissue origin are experimentally controlled (Cavanaugh, 1955; Sachs & DeHaan, 1973; Clay & DeHaan, 1979).

We have previously reported voltage-clamp measurements of some of the membrane currents underlying spontaneous activity of aggregates derived from ventricular cells of hearts at different developmental ages (Shrier & Clay, 1980; Clay & Shrier, 1981*a*, *b*). In the present report we describe novel measurements of currents in the pacemaker range of membrane potentials for atrial cell aggregates and we compare the pacemaker mechanisms of the two cell types, using computer simulations based in part on our previous measurements

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for ventricle cells and the present results for atrial cells. Furthermore, we demonstrate the current component responsible for the observed variability in the spontaneous beat rate of these preparations.

Materials and Methods

Aggregates of cells derived from the atria of 10 to 14-day-old hearts and from the apical portions of the ventricles of 12-day-old hearts of White Leghorn chicks were prepared using methods previously described (Sachs & DeHaan, 1973; Clay, DeFelice & DeHaan, 1979; Clay & Shrier, 1981*a*). Recordings of both the electrical activity and the underlying pacemaker currents were performed with a two-microelectrode current and voltage-clamp technique which has also been described (Clay & Shrier, 1981*a*). The external potassium ion concentration used in these experiments was 1.3 mM. The temperature was 37 °C maintained constant to within ±0.5 °C. Tetrodotoxin (TTX) at a final concentration of 3×10^{-6} M was usually added to the tissue culture dish prior to voltage-clamp measurements. Computer simulations of pacemaker voltage changes were carried out using the mathematical expressions given below (Results). The figures of the simulations were prepared with a Tektronix 4010 terminal and a 4631 Hard Copy Unit. All of the computations were carried out on a DEC PDP-11/60 digital computer located in the Laboratory of Biophysics, IRP, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, Mass.

Results

Spontaneous Activity

Aggregates of 12-day-old ventricular cells and 10 to 14-day-old atrial cells were usually observed to beat spontaneously and rhythmically. Roughly 10% of all preparations were quiescient. The beat rate of spontaneously active atrial preparations 180 to 220 μm in diameter ($n=10$) ranged between 13 and 100 beats per minute (b/min). There did not appear to be a relationship of activity with developmental age within the 10 to 14-day incubation period. The range of beat rates for comparable size 12-day ventricular preparations was 14 to 42 b/min ($n=9$). The variability of these data preclude any statistically significant comparison of the intrinsic beat rates of the two cell types. We did observe beat rates in atrial preparations which were considerably faster than any of our observations from ventricle. We also observed that the beat rate in atrial aggregates appeared to be more sensitive to impalement-induced leakage current than ventricular aggregates. We often observed beat rates of 5–10 b/sec in atrial preparations during and after-electrode impalement, whereas the fastest rates of induced beating in ventricular aggregates was 1–2 b/sec.

Representative electrical recordings from both atrial and ventricle preparations are shown in Fig. 1.

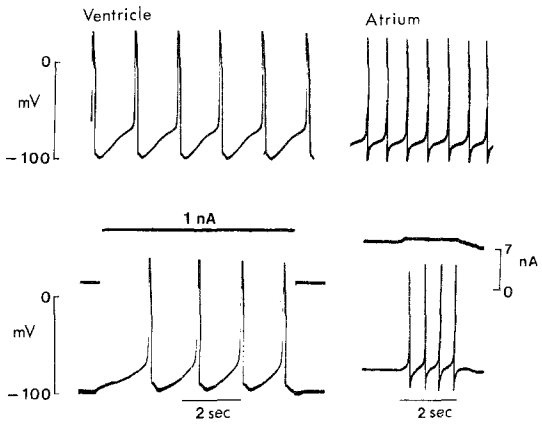

Fig. 1. Electrical activity of ventricle and atrial preparations. The top panels are from spontaneously active preparations; the bottom panels are from quiescent preparations. Current injected into the latter preparations induced activity as shown. In the case of the quiescent atrial preparation approximately 7 nA of steady depolarizing current was continuously injected during the initial phase of the record. The slight increase of injected current shown above the voltage recording was sufficient to induce firing

Quiescent aggregates could be induced to beat by injection of 1 to 10 nA steady depolarizing current, as shown in the bottom panels of Fig. 1. No significant difference was observed between the action potential parameters of spontaneous and induced activity for each tissue type. The parameters of ventricle action potentials were (Clay & Shrier, 1981*b*) 177±18 msec for the action potential duration (APD), −89±5 mV for the maximum diastolic potential (MDP, taken from the faster phase of repolarization of these action potentials), 26±4 mV for the overshoot potential (OS) and 142±36 V/sec for the maximum upstroke velocity (MUV, $n=5$). The corresponding parameters for the atrial action potentials were ADP=82±6 msec, OS=23±5 mV, MDP=−87±4 mV, MUV=106±25 V/sec ($n=9$). The only statistically significant difference in these results between the two tissue types is in the action potential duration. This difference is shown more clearly in Fig. 2, which is a superposition on an expanded time scale compared to Fig. 1 of action potentials from an atrial and a ventricular preparation.

The other clear tissue difference in the records of Fig. 1 is the shape of pacemaker depolarization. Ventricular aggregates show a secondary phase of hyperpolarization, which is clearly distinguishable from the repolarization phase of the action potential. The preparation then depolarizes to threshold. Prior to the rapid upstroke phase, the membrane potential often has a humped shape, as shown in the upper left panel of Fig. 1. The shape of pacemaker depolarization in atrial preparations is markedly different. These aggregates rapidly de-

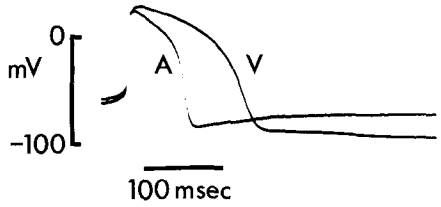

Fig. 2. Superimposed action potentials from spontaneously active atrial (*A*) and ventricle (*V*) aggregates

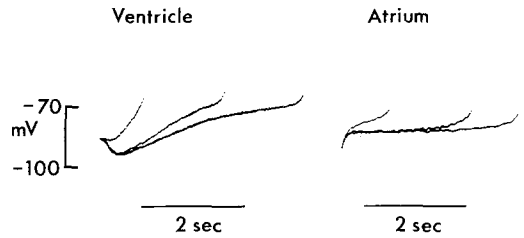

Fig. 3. Superimposed records of pacemaker voltage changes from a single ventricle and atrial preparation subsequent to electrode impalement, as described in text

polarize after each beat from MDP to a plateau region of pacemaker potentials. They subsequently depolarize at a slower rate until threshold is reached. Further examples of pacemaking are shown on a slightly expanded scale in Fig. 3. Each panel of this Figure consists of superimposed records from a single ventricle and atrial aggregate at three different points in time within a minute following electrode impalement. The left-hand record of each panel was taken immediately after impalement; the longest duration records were taken about one minute later. The interbeat interval was approximately 4 sec for each preparation. The differences between the records in each panel of Fig. 3 demonstrate the influence of leakage, or depolarizing current. We first note the lack of a significant effect of leak on MDP, which is consistent with our observations during controlled injection of depolarizing current of several nA or less. Currents of this amplitude do influence pacemaking; the secondary phase of hyperpolarization of ventricle preparations is reduced, or eliminated, and the humped shape of the pacemaker is similarly affected. In contrast, the qualitative shape of the atrial pacemaker is unaffected by depolarizing current.

Our final example of spontaneous activity, shown in Fig. 4, is from a ventricular aggregate which was beating intermittently. Occasionally, it ceased beating, as shown in Fig. 4. During this record the hump prior to each beat became more pronounced and, after the last beat, the membrane potential oscillated about -80 mV, where it came to rest after several seconds. We did not observe similar oscillations in atrial preparations.

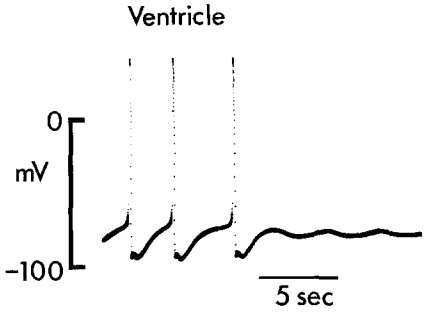

Fig. 4. Spontaneous activity and subthreshold oscillations of a ventricular aggregate. This particular preparation exhibited intermittent activity. The above recording demonstrates a typical cessation of activity

Voltage Clamp Analysis

Aggregates from 12-day-old ventricles exhibited two components of time-dependent current during voltage-clamp analysis; a potassium ion current activated in the -90 to -70 mV range of membrane potentials (I_{K_2}, *see* Discussion), and a current activated at potentials positive to -65 mV (I_x) whose ionic constituents are, as yet, unknown (Clay & Shrier, 1981*b*). Examples of I_{K_2} and I_x records from a single preparation in TTX at two different holding potentials, -58 and -80 mV, are shown in Fig. 5. The depolarizing step to -51 mV shows a clear time-dependent response, due to I_x, with a time constant of a few seconds. Upon return to -58 mV, the current relaxes with a time constant of approximately 1 sec. We also note the lack of an instantaneous current jump in this record, which suggests a lack of voltage dependence of the fully activated I_x current voltage relation ($\bar{i}_x$) in the -60 to -50 mV range. That is, even though some of the I_x channels are activated during the depolarizing step to -51 mV, they do not produce an instantaneous current jump upon return to -58 mV, because current flow through the channels is nearly the same at both -51 and -58 mV. This result is consistent with models of $\bar{i}_x$ (McAllister, Noble & Tsien, 1975; Beeler & Reuter, 1977) with a slight negative shift of $\bar{i}_x$ reversal potential. The hyperpolarizing step to -65 mV shows a smaller time-dependent current with a time constant of about 1 sec. The time constant of the tail, or "off" current upon return to holding potential, was approximately 2 sec. The records of hyper- and depolarization from -80 mV demonstrate the I_{K_2} component. The maximum time constant of this current is about 2 sec, which occurs in the -80 to -75 mV range. A complete description of I_{K_2} is given in Clay and Shrier (1981*b*).

Aggregates of 10- to 14-day atria appear to lack I_{K_2}. They do possess an I_x component. Voltage-

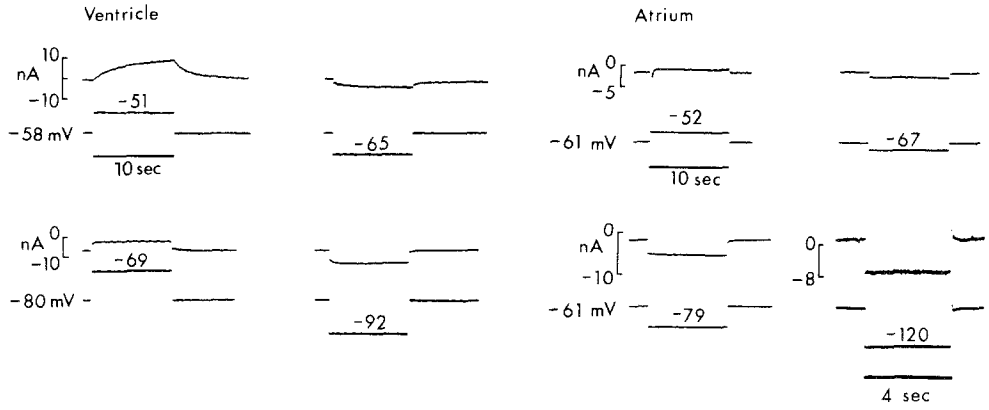

Fig. 5. Voltage-clamp analysis of ventricle and atrial preparations as described in the text. All current records are filtered by a low pass filter with 3 db cutoff at 100 Hz. Potential and current amplitudes as shown. The records from ventricle were taken from a single preparation in TTX from two different holding potentials, −80 and −58 mV. The atrial records to −52, −67 and −79 mV are from a single 10-day preparation in TTX. The −120 mV record is from a 12-day preparation which was not bathed in TTX. The duration of this step was 4 sec, as indicated. All other steps were 10 sec in duration

clamp records from a 10-day and a 12-day preparation are shown in Fig. 5 with a holding potential of −61 mV for each aggregate. The steps to −52, −67 and −79 mV are from the 10-day preparation (in TTX); the step to −120 mV is from the 12-day preparation (without TTX). The step to −52 is representative of the I_x component in these preparations. (This record may also contain an inward component presumably due to the slow inward current I_{si}.) This record and the corresponding result for ventricle illustrate one of the striking differences between the two tissue types; the I_x component in atria is activated at more positive potentials as compared to ventricle; its amplitude is markedly smaller; and its kinetics are markedly faster. The step to −79 mV and the tail current upon return to −61 mV both contain a small amplitude of time-dependent current (*see* Discussion). This result represents the largest time dependence in the −120 to −60 mV range in all of our measurements from 10- to 14-day-old atrial cells ($n=7$). The step to −120 mV is more representative of our results in this regard. We note that upon return to holding potential from −120 mV our voltage-clamp circuit was unable to prevent this preparation from firing. The action potential is not apparent in Fig. 5 because of the time scale. (Following the action potential the current trace reveals an apparent time dependence lasting about 1 sec. However, the voltage trace is also changing with a similar time dependence, but in the opposite direction. This pattern, in our hands, is an indication of inadequate voltage control of the preparation by the voltage-clamp circuit.)

Voltage-clamp analysis also reveals a difference between the two cell types in their steady-state current-voltage relations (*IV*'s). Representative *IV*'s are shown in Fig. 6 for quiescent (*Q*) and spontaneously active (*S*) ventricle and atrial preparations. The *IV*'s for ventricle have a nonlinear voltage-dependent shape with a region of negative slope conductance between −95 and −80 mV. This result is due primarily to the background current; I_{K_2} contributes at most 1 nA to the steady state *IV* (Clay & Shrier, 1981*b*). The marked increase in outward current at potentials positive to −70 mV is due in part to the steady-state amplitude of I_x. In contrast, the *IV*'s from atrial preparations are approximately linear (slope resistance $\simeq 100\ \mathrm{K\Omega\,cm^2}$). There is virtually no contribution to these *IV*'s from time-dependent components. We further note the lack of any significant difference in the relative voltage-dependent shape of the *IV*'s from spontaneously active and quiescent preparations. This result provides an important clue to understanding the variability in the beat frequency results (Discussion).

Simulation of Pacemaker Voltage Changes

We have simulated the pacemaker process from mathematical descriptions of our measurements of background and time-dependent pacemaker current and the measurements of the TTX sensitive, inward sodium current by Ebihara, Shigeto, Lieberman and Johnson (1980) and Ebihara and Johnson (1980). The parameters of our model are scaled for a 200-μm diameter aggregate, which has approximately $2.15\times10^{-2}\ \mathrm{cm^2}$ of membrane surface area (Clay & Shrier, 1981*a*). All currents are given in units of nA, voltages in mV.

We describe the background current of atrial preparations, $I_{bg,A}$, by the straight line

$$I_{bg,A}=0.2(V+50). \quad (1)$$

The nonlinear $I_{bg,V}$ component of ventricle is given by (Clay & Shrier, 1981*a*, *b*)

$$I_{bg,V}=I_{K_1}+I_{Nab} \quad (2)$$

where

$$I_{K_1}=300(q/p)^6(p-q)/(1+y+y^2+y^3+y^4+y^6) +0.25(V+50)/(1-\exp(-(V+50)/25)) \quad (3)$$

where

$$p=(1+\exp(-(V+100)/25))^{-1};\ q=1-p;\ y=q/p \quad (4)$$

and

$$I_{Nab}=0.07(V-40). \quad (5)$$

The I_x component in ventricular cell aggregates is given by

$$I_x=100s_x(t) \quad (6)$$

with

$$ds_x(t)/dt=-(\alpha_x+\beta_x)s_x(t)+\alpha_x \quad (7)$$

where

$$\alpha_x=0.04(V+10)/(1-\exp(-(V+10)/10)\ \sec^{-1}$$
$$\beta_x=0.01\exp(-(V+10)/10)\ \sec^{-1}. \quad (8)$$

The steady-state I_x current together with $I_{bg,V}$ satisfactorily describes the voltage-dependent shape of the ventricle *IV*'s in Fig. 6. The potential at which $I_{bg,V}+I_x=0$ is -61 mV, which closely approximates the zero-current potential of the lower *IV* in the left-hand panel of Fig. 6. The first term in the expression for I_{K_1} in Eq. (3) is derived from a single file diffusion model of inward rectification in ionic channels (Clay & Shlesinger, 1977; J.R. Clay and M.F. Shlesinger, *unpublished*). The second term in Eq. (3) is derived from the McAllister, Noble, Tsien (1975) model of i_{K_1} in Purkinje fibers. The model of I_x in Eqs. (6)–(8) was chosen to describe the time-dependent current positive to -65 mV in 12-day preparations. The α_x and β_x parameters in Eq. (8) are modifications of our α_s and β_s I_{K_2} parameters (Clay & Shrier, 1981*a*, *b*) chosen to fit our measurements of I_x time constants. The I_{K_2} component in these preparations is given by (Clay & Shrier, 1981*b*)

$$I_{K_2}=100s(t)(z/w)^2(w-z)/(1+x+x^2) \quad (9)$$

with

$$w=(1+\exp(-(V+124)/25))^{-1};\ z=1-w;\ x=z/w \quad (10)$$

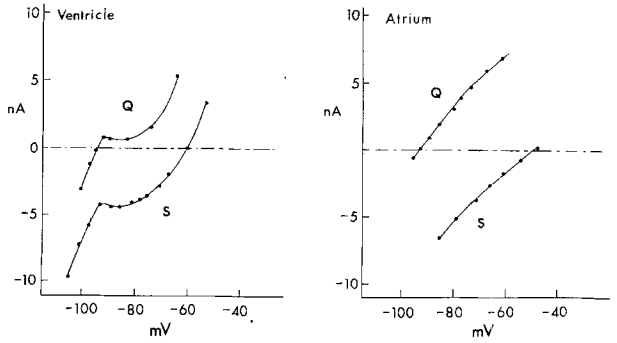

Fig. 6. Steady-state current-voltage relations (*IV*'s) for spontaneously active (*S*) and quiescent (*Q*) atrial and ventricular preparations. Currents measured either at the end of 10-sec clamp steps or in steady holding potential conditions in the presence of TTX

and

$$ds(t)/dt=-(\alpha_s+\beta_s)s(t)+\alpha_s \quad (11)$$

with

$$\alpha_s=1.8(V+61)/(1-\exp(-0.28(V+61)))\ \sec^{-1}$$
$$\beta_s=0.007\exp(-0.21(V+61))\ \sec^{-1}. \quad (12)$$

The TTX-sensitive, fast inward sodium current I_{Na} is given by

$$I_{Na}=323m^3(t)h(t)(V-40) \quad (13)$$

with

$$dm(t)/dt=-(\alpha_m+\beta_m)m(t)+\alpha_m$$
$$dh(t)/dt=-(\alpha_h+\beta_h)h(t)+\alpha_h \quad (14)$$

where

$$\alpha_m=100(V+45)/(1-\exp(-0.1(V+45)))\ \sec^{-1}$$
$$\beta_m=4\times10^3\exp(-(V+70)/18)\ \sec^{-1}$$
$$\alpha_h=135\exp(-(V+80)/6.8)\ \sec^{-1}$$
$$\beta_h=3.56\times10^3\exp(0.079V)+3.1\times10^8\exp(0.35V)\ \sec^{-1}. \quad (15)$$

The expression for I_{Na} in Eq. (13) is of similar form as the Hodgkin and Huxley (1952) model for I_{Na} in nerve. The expressions for α_m and β_m in Eq. (15) are the same as in their model except for a 10-mV shift of potential in the negative direction. The expressions for α_h and β_h are taken from Ebihara and Johnson (1980).

Pacemaker voltage changes were simulated from

$$dV/dt=-(I_{ionic}+I_{app})/C \quad (16)$$

where C is the input capacitance (approximately 0.023 μf for a 200 μm diameter aggregate (Clay et al., 1979)). I_{app} is the injected current, and $I_{ionic}=I_{bg,A}$

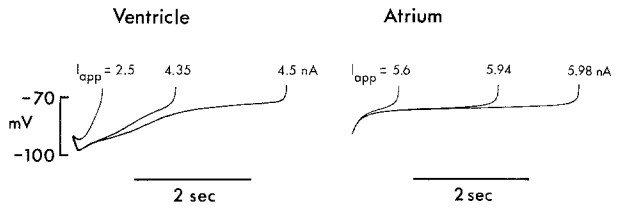

Fig. 7. Simulations of ventricle and atrial pacemaker voltage changes as described in the text

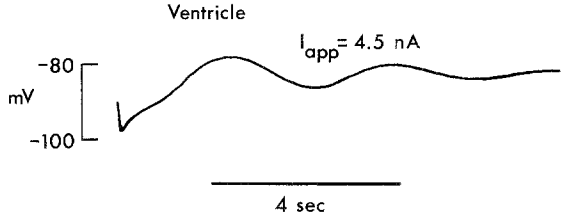

Fig. 8. Simulation of subthreshold voltage oscillations in ventricle. The oscillations are attributable to I_x and I_{Na}

$+I_{Na}$ for atrial preparations and $I_{bg,V}+I_{Na}+I_{K_2}+I_x$ for ventricle preparations. The initial condition for all simulations was $V=-90$ mV, which closely approximates MDP both for atria and ventricle. We also set the activation parameters s and s_x initially equal to 1, since they are fully activated at the peak of the action potential and there is insufficient time during repolarization for them to change significantly. We initially set $m=0$ and $h=1$, which closely corresponds to the steady-state values of these parameters at MDP. This choice of initial conditions for I_{Na} is appropriate, since the duration of repolarization is significantly longer than either τ_m or τ_h. The time course of voltage change from these initial conditions was determined by numerically integrating Eq. (16) using the algorithm of Rush and Larsen (1978) with a 50 μsec integration time step. Each simulation was terminated after a specified time limit or after the potential crossed threshold (arbitrarily chosen to be −65 mV). The time from MDP to threshold of the ventricle model with $I_{app}=0$ was 0.157 sec; that of the atrial preparation was 0.107 sec. These results correspond to *IV*'s which cross the voltage axis at the positive end of our observations (−50 mV for atria; −61 mV for ventricle). They also give beat rates which are the highest that we have observed. Results for $I_{app}>0$ are shown in Fig. 7. (Adding an outward applied current to the model is equivalent to shifting the *IV* in the positive direction.) The ventricle model with $I_{app}=2.5$, 4.45 and 4.55 nA gives times from MDP to threshold of 0.487, 1.72 and 3.59 sec, respectively. Similarly, $I_{app}=5.6$, 5.94 and 5.98 nA in the atrial model gives times from MDP to threshold of 0.785, 2.47 and 3.98 sec, respectively. These values of I_{app} were chosen to give interbeat intervals roughly comparable to the experimental recordings in Fig. 3. We note that the simulations qualitatively mimic all of the features of pacemaking (*see* Discussion). The ventricle simulations show an additional inflection near $V=-90$ mV, which we have observed in some preparations. Finally, we note that both the atrial and ventricular models fail to reach threshold for sufficiently positive values of I_{app} (6 nA or greater for atria, 4.5 nA or greater for ventricle). The ventricle model exhibits oscillations when it fails to cross threshold, as shown in Fig. 8 ($I_{app}=4.5$ nA). This result is to be compared with the experimental observations of oscillations shown in Fig. 4. The atrial model does not exhibit similar oscillations.

Discussion

We have described differences in electrical activity in embryonic atrial and ventricular heart cells which are explicable in terms of the underlying membrane currents. Specifically, spontaneous atrial preparations depolarize from MDP to a plateau region of pacemaker potentials with a time constant (~100 msec) which is equal to the product of membrane capacitance and slope resistance of I_{bg}. The membrane then slowly depolarizes to threshold. In contrast, spontaneous ventricular preparations have a more complex pacemaker depolarization. The initial hyperpolarization subsequent to MDP is caused by I_{K_2}, which is an outward current with a negative slope *IV*. The I_{K_2} term is fully activated (i.e., $s=1$) at MDP. Consequently, the net *IV* is outward at this point and it also has negative slope. Therefore, the potential hyperpolarizes until the s variable deactivates, thereby allowing the membrane to be depolarized by I_{bg} and I_{app}. The I_{K_2} term does not contribute further to the pacemaker beyond the first 0.5 sec after MDP. The inflection of depolarization near the 1-sec mark of the $I_{app}=4.96$ nA simulation in Fig. 7 is due to the fact that $I_{bg}+I_{app}$ is only slightly greater than zero at this point ($V=-90$ mV). The I_{K_2} term also does not contribute to the oscillations shown in Fig. 8. This result is due to the I_x and I_{Na} components, in contrast to 7-day ventricle preparations and Purkinje fibers, both of which exhibit subthreshold oscillations due, in part, to I_{K_2} (Vassalle, 1965; McAllister et al., 1975; Guevara, Shrier, Clay & Glass, 1982). Finally, we note that I_{K_2} in Purkinje fibers has recently been reinterpreted as an inward current component, rather than an outward potassium ion component (DiFran-

cesco, 1981; DiFrancesco & Noble, 1982). An important part of this alternative view is a significant potassium ion accumulation in the intercellular cleft spaces. The issue of ion accumulation in aggregates is not entirely settled, although our measurements of I_{K_2} kinetics in the vicinity of E_K suggests that it may not be significant during voltage-clamp measurements in the pacemaker voltage range (Clay & Shrier, 1981*a*). In any case, the I_{K_2} issue is not central to the present analysis. The I_{K_2} kinetics play a role only during the early phase ($\sim$0.5 sec) of pacemaking in 12-day ventricle cells, and they appear to be absent from 10- to 14-day atrial cells.

The final $\sim$2 sec of depolarization in both the $I_{app}=4.5$ nA (atrium) and the $I_{app}=5.98$ nA (ventricle) simulations in Fig. 7 is due to I_{Na} and the slope of the IV near threshold. The I_{Na} component is known to play a role in cardiac pacemaking. For example, the increase in I_{Na} during the final 300 msec of the normal Purkinje fiber action potential is comparable to the change in I_{K_2} during this phase (McAllister et al., 1975). Our analysis of normal pacemaking in 7-day ventricle cells is similar to theirs (Guevara et al., 1982). The present analysis differs in that I_{K_2}, or other time-dependent currents with 1- to 2-sec kinetics, do not contribute to the final $\sim$2 sec of pacemaking in the examples of Fig. 7. A similar example of long firing intervals with respect to the kinetics of the underlying currents exists in the neurophysiology literature. Encoder neurons are known to fire in a stable manner at frequencies as low as 1 to 2 spikes/sec (Fuortes & Mantegazzini, 1962; Jack, Noble & Tsien; 1975), which is puzzling, since the Hodgkin and Huxley (1952) model of the nerve action potential predicts a minimum firing frequency of about 100 spikes/sec (Conner, Walter & McKown, 1977). Several attempts have been made to describe encoder neurons by modifying the potassium ion conductance in the Hodgkin and Huxley (1952) model, or by adding to the model a conductance with slow kinetics (references cited in Connor et al. (1977)). This issue was resolved by Connor et al. (1977), who demonstrated with computer simulations that a transient potassium conductance with 5-msec kinetics, which is observed in molluscan neurons, can produce firing intervals of several hundred milliseconds provided that the dynamic and steady-state features of the conductance were appropriately tuned, and that the slope resistance of the steady state IV near threshold was high. Connor et al. (1977) noted that I_{Na} could produce a similar effect, although they did not pursue this issue, since it appears to be inappropriate for neurons. Our analysis represents an extension of their work, since we found that the steady-state amplitude of I_{Na} together with a high resistance background IV can produce firing intervals of a few seconds duration, or even longer. The slope resistance of our preparations in TTX is considerably higher than that of nerve cells; 100 to 200 KΩ cm^2 near threshold. The steady-state m and h curves of the I_{Na} conductance overlap in this voltage range. Therefore, the m^3h term in Eq. (13) is not zero at these potentials. Moreover, the IV of this component has negative slope. Consequently, the total IV is flattened still further near threshold compared to TTX conditions. That is, the slope resistane is even greater than 200 KΩ cm^2, which suggests that the effective RC time constant can be in the range of several seconds provided that I_{bg} is at the appropriate level. Our simulations have demonstrated the feasibility of this mechanism. Of course, simulations alone provide only sufficient proof for a theoretical scheme. Moreover, the voltage-clamp records for atrium in Fig. 5 do provide a hint of time dependence in atrial preparations, which we did not include in our simulations. The record from -61 to -79 mV in Fig. 5 shows a slight time dependence immediately following the voltage step and a more pronounced time dependence following return to -61 mV. This measurement was from a 10-day preparation, the earliest in the present investigation. Therefore, the time dependence may reflect the final stage of loss of conductance, similar to the loss of I_{K_2} in ventricle cells (Shrier & Clay, 1980; Clay & Shrier, 1981*b*). The -79 mV record also shows a slight drift in the inward current direction throughout the duration of the record which, in our hands, is an occasional artifact of two-microelectrode voltage-clamp recording. The drift in the -120 mV record is less and, if anything, slightly in the outward direction. As noted above, the tail current time dependence in this record is related to loss of voltage control upon return to -61 mV. We did not observe a significant inward time dependence in atrial cell aggregates similar to the results of Brown and DiFrancesco (1980) on rabbit sino-atrial tissue. Moreover, we believe that the slight time dependence we did observe does not play a significant role in pacemaking, as revealed by our computer simulations and by small signal impedance measurements. We did not observe small signal oscillations in the -90 to -60 mV range, in contrast to Purkinje fibers (Trautwein & Kassebaum, 1961), 7-day ventricle (Clay et al., 1979), and the atrial preparations themselves at potentials positive to -50 mV. Consequently, we feel that our model of pacemaking both in the atrial and ventricle cell aggregates is appropriate.

Our results demonstrate that the interbeat in-

terval is produced by a touchy balance of membrane currents. The variability in the beat rate observations described above is, therefore, not surprising, since the interbeat interval is the most labile physiological parameter of the system. The results of Fig. 6 indicate that a component of I_{bg} is primarily responsible for the variability. The fact that the shapes of the *IV*'s are approximately the same for both quiescent and spontaneously active preparations suggests that the difference between these results is due either to a nonspecific leakage current or to the background sodium current, $I_{\mathrm{Na},b}$. The variation in absolute conductance which underlies the difference between pacemaking and nonpacemaking preparations is relatively small. The difference between the *IV*'s in either Fig. 6*A* or 6*B* is only 10 nA which is equivalent to a change in background sodium conductance ($\bar{g}_{\mathrm{Na},b}$) of only 0.005 mS cm^{-2}. In contrast, the peak conductance of I_{Na} is $\sim$20 mS cm^{-2} (Ebihara & Johnson, 1980). Consequently, we conclude that relatively small changes in pacemaker current can produce significant modifications in spontaneous activity. Therefore, the absolute rate of beating of embryonic cells should be used with caution as a determinant of underlying pacemaker mechanisms.

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