An ionic model for rhythmic activity in small clusters of embryonic chick ventricular cells

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An ionic model for rhythmic activity in small clusters of embryonic chick ventricular cells. Am J Physiol Heart Circ Physiol 289: H398–H413, 2005. First published February 11, 2005; doi:10.1152/ajpheart.00683.2004.—We recorded transmembrane potential in whole cell recording mode from small clusters (2–4 cells) of spontaneously beating 7-day embryonic chick ventricular cells after 1–3 days in culture and investigated effects of the blockers D-600, diltiazem, almokalant, and Ba2+. Electrical activity in small clusters is very different from that in reaggregates of several hundred embryonic chick ventricular cells, e.g., TTX-sensitive fast upstrokes in reaggregates. The slow upstroke is generally lower in single cells and in clusters. The upstroke velocity is much lower in single cells, in small clusters of cells, and in sparse monolayers than in reaggregates. Spontaneous beating can be abolished by addition of tetrodotoxin (TTX), a blocker of the fast inward Na+ current (INa), to the medium bathing reaggregates of TTX-insensitive 7-day embryonic ventricular cells, but spontaneous activity continues in single cells and monolayers. However, spontaneous activity in some TTX-sensitive preparations can be abolished by addition of either of the Ca2+ channel blockers D-600 or diltiazem.

We carried out an experimental study to characterize the electrical properties of spontaneously beating clusters of cells isolated from the 7-day embryonic chick ventricle and then assembled a mathematical model of this activity. The goal is to use this model to investigate phenomena seen in experiments, such as irregularity of beating in small clusters, mutual synchronization of pairs of cells, phase resetting and phase locking, current-pulse-induced annihilation of spontaneous activity, and termination of spiral-wave reentrant motions in monolayers. Although ionic models of reaggregates of embryonic chick atrial cells have been described, we are not aware of any models of small clusters of isolated embryonic chick ventricular cells.

METHODS

Cell Isolation

Ventricular myocytes were isolated from embryonic chick hearts by means of techniques previously described (50) with modifications (79). The hearts of 7-day embryos were removed, and the ventricles were chopped off, minced, and transferred to flasks containing 0.25% trypsin (bovine pancreas; Boehringer Mannheim, Germany) in a nominally Ca2+- and Mg2+-free Hanks’ balanced salt solution (HBSS; in mM: 137 NaCl, 5.4 KCl, 0.34 Na2HPO4, 0.44 KH2PO4, 4.2 NaHCO3, and 5 glucose, pH 7.4). The flasks were placed in a shaker bath at 37°C for 7 min. The resulting cell suspension was gently agitated with a pipette and filtered through a 100-μm mesh. HBSS, supplemented with fetal calf serum (5% final concentration), was added to the cell suspension to reach a concentration of 106 cells/ml. The cell suspension was then plated in 35-mm culture dishes (Mentei) and incubated in a shaker bath at 37°C. After 2 days in culture, the cell suspension was gently aspirated and replaced with fresh HBSS. After a couple of days in culture, the electrical activity in an isolated embryonic chick ventricular cell, in a small cluster of a few such cells, or in a sparse monolayer is very different from that in situ or in a reaggregate of hundreds or thousands of cells isolated from the ventricle. For example, when trypsin-dispersed ventricular cells from 7-day embryonic chick hearts are used, the upstroke velocity is much lower in single cells, in small clusters of cells, and in sparse monolayers than in reaggregates. Spontaneous beating can be abolished by addition of tetrodotoxin (TTX), a blocker of the fast inward Na+ current (INa), to the medium bathing reaggregates of trypsin-dispersed 7-day ventricular cells, but spontaneous activity continues in single cells and monolayers. However, spontaneous activity in some TTX-insensitive preparations can be abolished by addition of either of the Ca2+ channel blockers D-600 or diltiazem.

We carried out an experimental study to characterize the electrical properties of spontaneously beating clusters of cells isolated from the 7-day embryonic chick ventricles and then assembled a mathematical model of this activity. The goal is to use this model to investigate phenomena seen in experiments, such as irregularity of beating in small clusters, mutual synchronization of pairs of cells, phase resetting and phase locking, current-pulse-induced annihilation of spontaneous activity, and spontaneous initiation and termination of spiral-wave reentrant motions in monolayers. Although ionic models of reaggregates of embryonic chick atrial cells have been described, we are not aware of any models of small clusters of isolated embryonic chick ventricular cells.

SPONTANEOUS ACTIVITY based on generation of the pacemaker potential (spontaneous phase 4, or diastolic, depolarization) is not normally found in adult ventricular muscle in situ, nor is it normally found in single cells freshly isolated from adult ventricular muscle. In contrast, early enough during development, ventricular muscle (or areas of the heart destined to eventually become ventricular muscle) can beat spontaneously (1, 97). Spontaneous electrical activity can also be seen in single cells and in small clusters of cells isolated from the embryonic chick ventricle (10, 17, 26, 49, 51, 78, 95), in the embryonic mouse ventricle (117), and in the neonatal rat ventricle (86).
concentration), was added to stop trypsin activity. The cell suspension was centrifuged at ~100 g for 5 min at 4°C, the supernatant was discarded, and the cell pellet was resuspended in fresh trypsin-free HBSS. The centrifugation and resuspension processes were then repeated. The solution was centrifuged for a third time and resuspended in cell culture medium [M199 (Sigma) supplemented with 4% fetal calf serum, 2% horse serum, and 0.7 mM glutamine, pH 7.4] to yield a density of 5 × 10^3 cells/ml.

The cell suspension was separated into aliquots on nonadhesive plastic culture dishes that were incubated at 37°C in a water-saturated atmosphere of 95% air-5% CO2. To obtain small clusters of cells, 0.6-ml aliquots of cell suspension were removed after 0.5–2 days and placed into the lumen of flexiPERM silicone rings (Heraeus, Hanau, Germany), which were attached to microscope slide coverslips. Coverslips with attached flexiPERM rings were placed in cell culture dishes (Greiner) and stored in the incubator. This procedure allowed the myocytes to adhere to the glass surface, where they could divide and form small clusters of cells. However, we can offer no guarantee that a particular cluster is made up entirely of cells that divided in culture.

Experiments were performed 2–36 h after the cells were plated, on clusters containing two to four cells. Recordings were made in the whole cell mode from clusters that were spontaneously beating before they were patched. We use clusters of a few cells, rather than single cells, because we were unable to obtain recordings of spontaneous activity from single cells. [It is also easier to successfully impale a cell in a cluster, rather than a single cell, if a conventional sharp microelectrode is used (17, 26).] In addition, the effect of the seal-leak current (Iseal; see below) on spontaneous activity is expected to be considerably smaller for a cluster than for a single cell (but see Ref. 78). For electrophysiological recording, the coverslip with attached myocytes was used to form the bottom of the experimental chamber, which was placed on the stage of an inverted microscope (Zeiss).

The transmembrane potential (V) was recorded using the whole cell recording mode of the patch-clamp technique. Patch pipettes (~2 MΩ resistance) were pulled from glass capillary tubes and filled with pipette solution (in mM: 137 NaCl, 5.4 KCl, 1.8 CaCl2, 1.1 MgCl2, 2.2 NaHCO3, 0.4 NaH2P04, 10 Na-HEPES, and 5.6 glucose, with pH adjusted to 7.4 with NaOH) at 36–37°C with a flow rate of 1.5 ml/min.

**Electrophysiological Recording**

**Action Potential Parameters**

Several parameters are measured to characterize spontaneous electrical activity. Interbeat interval (IBI) is the time between consecutive crossings of 0 mV on the upstroke of the action potential. The maximal diastolic potential (MDP) is the most negative voltage recorded during an action potential and the overshoot potential (OS) the most positive. The action potential amplitude (APA) is the difference between MDP and OS. Action potential duration (APD) is the time from the crossing of 0 mV on the upstroke to the time of 50% repolarization (APD50) or 100% repolarization (APD100). The dastaic depolarization rate (DDR) is the slope of the chord joining the point where (MDP + 1 mV) is crossed and the point 70 ms later (108, 114). The maximum rate of rise of the upstroke (Vmax) is computed using the greatest voltage difference between two consecutive samples on the upstroke (1-ms sampling interval).

**Simulation Methods**

Numerical integration of the Hodgkin-Huxley-type ionic model was carried out using a forward Euler scheme, with V at time t + Δt calculated as follows

\[ V(t + \Delta t) = V(t) - (\Delta t/C_m) \sum I_i(t) \]

where \( C_m \) is membrane capacitance and \( I_i \) represents the individual ionic currents described below. (A 4th-order Runge-Kutta integration scheme produced virtually superimposable results.) With our choice of time step (\( \Delta t = 0.1 \text{ ms} \)), the change in voltage from time t to t + \( \Delta t \) was kept to <1 mV. The value of each activation or inactivation variable (\( \xi \)) at time t + \( \Delta t \) was obtained from its value at time t using the analytic formula

\[ \xi(t + \Delta t) = \xi(\infty) - \left[ \xi(\infty) - \xi(t) \right] e^{-\Delta t/\tau} \]

where \( \xi(\infty) \) is the steady-state or asymptotic value of \( \xi \) at V(t) and \( \tau \) is the time constant of \( \xi \) at V(t). L’Hôpital’s rule was used to calculate \( \alpha_n \) when V came to within ±0.1 mV of the value producing an indeterminate form. The numerical integration routine was written in C, and all variables were double precision (~16 significant decimal places).

Bifurcation analysis was carried out using AUTO, as incorporated in XPPAUT (25). The model equations file for use with XPPAUT is available as supplemental material (supplemental data for this article may be found at http://ajphpathophysiology.org/cgi/content/full/06683-4.2004.DC1).

**Formulation of the Model**

Our model consists of six currents: a slow inward Ca2+ current (Ica), a slow delayed K+ current (IKc), a rapid delayed rectifier K+ current (IKr), an inward rectifier K+ current (IK1), a linear time-independent background current (IB), and a linear nonspecific seal-lease current (Iseal), generated by the leakage of ions through the gigaseal seal of the recording pipette. We now give the rationale for including each of these currents and for the particular formulation that we employ for each of these currents, as well as reasons for using a “first-generation,” rather than a “second-generation,” model.

**Difficulties with second-generation models.** In the earlier Hodgkin-Huxley-type ionic models of cardiac cells, all the concentrations of the various ionic species were held fixed, so that no provision had to be made for pumps and exchangers to regulate these concentrations. We refer to models that incorporate both of these refinements as second-generation models, in contrast to the earlier first-generation models. We formulate our model below as a more primitive first-generation model, because there are two major problems with the more physiologically realistic second-generation models: 1) drift, with very slow long-term trends in some of the variables, particularly some ionic
concentrations (3, 21, 23, 24, 37, 45, 103, 116), and 2) degeneracy, with nonuniqueness of equilibrium solutions such as steady states and limit cycles (23, 24, 30, 37, 102).

Drift has been managed in several ways: 1) by finely adjusting parameters to achieve flux balance across the membrane (21, 2) by adding an electroneutral Na\(^+\) current of a precise size to produce stability of concentrations (74, 3) by monitoring the stimulus current in a paced quiescent cell (37, 45), and 4) by ensuring that certain ionic concentrations remain fixed (3, 55, 103, 121). It is not clear whether strategies 1 and 2 are robust, because a change in some parameter in the model might require further fine adjustment of the stabilizing parameters. (This is reminiscent of a neutrally stable equilibrium.) Strategy 3 is, of course, of no use in an unpaced pacemaker cell. Strategy 4 defeats, at least in part, the initial intent in formulating the model as a second-generation model; e.g., when all the internal and external iconic concentrations are held constant, the Na\(^+\)K\(^+\) pump current (\(I_{\text{NaK}}\)) and the Na\(^+\)/Ca\(^{2+}\) exchange current (\(I_{\text{NaCa}}\)) are effectively background currents, and one is left essentially with a first-generation model, in which activity-dependent effects due to changes in certain ionic concentrations are not manifest.

The other major problem noted with second-generation models is degeneracy. In second-generation models of different types of cardiac cells, the system of differential equations could be rewritten as a system of \(N - 1\) equations in \(N\) unknowns (30, 102). The Jacobian is then singular, and there is a continuum of equilibrium points, rather than isolated equilibrium point(s), so that, e.g., the resting potential of a quiescent system depends on the initial conditions (30, 102). A similar finding of degeneracy holds for the limit cycle that corresponds to spontaneous activity (24, 30). It has been suggested that the original \(N\)-variable fully differential model should be recast as a differential-algebraic system, with the equation controlling voltage being algebraic and the remaining \((N - 1)\) equations being differential (23, 24, 30, 37, 45, 102). In one report in a sinoatrial (SA) node model in which the differential-algebraic formulation was used, it was stated that there was no long-term drift (23). In earlier work in which drift was abolished by making some ionic concentrations fixed, this also had the unintended benefit of removing the degeneracy, thus allowing the bifurcation analysis of isolated equilibria by means of continuation techniques (103; see also Refs. 55 and 121).

Finally, in situations such as ours in which cells are studied using patch micropipettes, a more realistic model of the experimental situation is one in which internal concentrations are kept fixed as a result of Ca\(^{2+}\) buffering with EGTA and dialysis of the cell contents of a very small cell volume against the much larger pipette volume (55, 121). Making concentrations fixed then also removes degeneracy and drift. Given all the above uncertainties and complications and given that very little information is available about the control of intracellular ionic concentrations in our cells, we decided to use a first-generation model, as have some authors of other quite recent studies (3).

Capacitance. Unless stated otherwise, ventricular and atrial cells will refer to embryonic chick ventricular and atrial cells, respectively, and \(n\)-day will refer to a cell isolated from the embryo after \(n\) days of incubation. Because the capacitance of a single 7-day ventricular cell in our laboratory is 8–9 pF, we set the capacitance in our three-cell model cluster to 25.5 pF. Our value of the single-cell capacitance in our laboratory is 8–9 pF, we set the capacitance in our three-cell incubation. Because the capacitance of a single 7-day ventricular cell

in only 45% of 7-day ventricular cells was \(I_{\text{Ca}}\) clearly separable into \(I_{\text{CaL}}\) and \(I_{\text{CaT}}\) on the basis of the voltage threshold for activation (44). In these cells, \(I_{\text{CaL}}\) is half-inactivated at \(-49\) mV, whereas \(I_{\text{CaT}}\) is half-inactivated at \(-27.5\) mV. In the 55% of cells with nonseparable \(I_{\text{Ca}}\), half-inactivation occurs at \(-42.8\) mV.

We have chosen to use a nonseparable description of \(I_{\text{Ca}}\) in our model

\[
I_{\text{Ca}} = g_{\text{Ca}}f(V - E_{\text{Ca}})
\]

where \(g_{\text{Ca}}\) is the maximal conductance, \(d\) is the activation variable, \(f\) is the inactivation variable, and \(E_{\text{Ca}}\) is the reversal potential, which we set to 40 mV on the basis of our own voltage-clamp results (79). The equations governing \(d\) and \(f\) are given in the appendix.

The steady-state inactivation \((f_d)\) curve of the nonseparable current in 7-day ventricular cells, which lies between the inactivation curves for \(I_{\text{CaL}}\) and \(I_{\text{CaT}}\), is taken from Fig. 9 of Ref. 44. Because the steady-state activation \((d_u)\) curve of the nonseparable current was not reported, we assume that it lies between the \(I_{\text{CaL}}\) and \(I_{\text{CaT}}\) activation curves of Fig. 10 of Ref. 44 and that it has an intermediate slope factor. We have chosen the values of parameters so that the \(d_u\) curve is closer to the \(I_{\text{CaL}}\) than to the \(I_{\text{CaT}}\) activation curve, in order that the peak-current current-voltage \((I-V)\) curve has a maximum at \(-60\) mV, which is close to the experimental value for the nonseparable current (44). Figure 1A shows the steady-state \(d_u\) and \(f_u\) curves.

There are no systematic reports of the time constants of activation \((\tau_a)\) and inactivation \((\tau_i)\) of the nonseparable current. We therefore take the expressions unchanged from Ref. 66, which describes \(I_{\text{CaL}}\) and \(I_{\text{CaT}}\) in a 37\(^\circ\)C guinea pig model. The \(\tau_a\) curve (Fig. 1B) has a typical bell shape, whereas the \(\tau_i\) curve (Fig. 1B) has the U shape that is seen in mammalian cells and in 7-day ventricular reaggregates (72).

We have set \(g_{\text{Ca}}\) so that \(V_{\text{max}}\) is 9.5 V/s in the model, which is close to our mean experimental result (8.5 V/s). The peak-current \(V-I\) curve then has a maximum value of \(-30\) pA/pF at \(-40\) mV (Fig. 1C), which is within the range seen in our cells (79); see also Fig. 4C of Ref. 44 and Fig. 1 of Ref. 13, scaled for differences in capacitance and

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Fig. 1. Characteristics of slow inward Ca\(^{2+}\) current (\(I_{\text{Ca}}\)) in the model. A: steady-state activation (\(d_u\)) and inactivation (\(f_u\)) curves. B: time constants of activation \((\tau_a)\) and inactivation \((\tau_i)\). C: peak \(I_{\text{Ca}}\) in voltage-clamp steps from a holding potential of \(-80\) mV to various test potentials (protocol of Ref. 44). D: simulated current during voltage-clamp steps from a holding potential of \(-80\) mV to \(-10\) mV and \(-20\) mV (protocol of Ref. 44).
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Also Fig. 7 to the corresponding experimental traces in Ref. 79 (see voltage-clamp protocol in the model are similar, in magnitude and D corresponding to those more recently termed atrial cells (9, 88, 89), in small clusters of ventricular cells (79), and for cell-cell contact-dependent regulation of expression of two differ-
ent K+ currents. The delayed K+ current (I_Kr) has been described (8). This value is depolarized to the equilibrium potential for K+ of −85 mV, computed from the Nernst equation at 37°C, external [K+] of 5.4 mM, and pipette [K+] of 129 mM. This relatively depolarized value of E_Kr has been found in many studies on I_Kr, and has been attributed to a slight permeability of the channel to Na+ (65, 121). In obtaining the formulas for the rate constants α and β (see APPENDIX), we have first divided the original equations (52) by a factor of 3, to obtain a τn curve consistent with the experimental values in single ventricular cells at room temperature (see Fig. 2C of Ref. 8), and then multiplied αn and βn by a factor of 2, in correspondence with the reported Q10 (111), to obtain values appropriate for our experimental temperature of 36–37°C (Fig. 2B). The maximal conductance (g_Kr) was set to give a fully activated I-V curve (Fig. 2C), similar to that seen experimentally (see Fig. 1C of Ref. 8), scaled for capacitance and temperature (Q10 ≈ 2) (111). The steady-state activation curve (Fig. 2A) and the voltage-clamp currents (Fig. 2D) are similar to those previously reported from our laboratory at 37°C (79), as well as those reported elsewhere (8), when compensated for temperature.

For I_Kc, we use the formulation

\[ I_{K_c} = g_{K_c} n(V - E_{K_c}) \]

where \( n \) is the activation variable, and we introduce \( z(V) \) to provide inward rectification so that the fully activated I-V relation (Fig. 3C) is similar in shape to that seen in atrial reaggregates (see Fig. 8C of Ref.

Delayed K+ currents. The delayed K+ currents I_Kc and I_Kr have been described in reaggregates of ventricular cells (13, 14, 88) and atrial cells (9, 88, 89), in small clusters of ventricular cells (79), and in single ventricular (8) and atrial (12) cells. These currents have also been observed at the single-channel level in ventricular cells (8, 67).

Two components of the delayed current, initially termed I_x1 and I_x2, have been seen in atrial reaggregates (89). These two currents correspond to those more recently termed I_Kc and I_Kr, respectively, in isolated adult mammalian ventricular cells. I_x2, or I_Kr, has been described in 7-day ventricular reaggregates (14, 15), in single ventricular (8), and in small clusters of such cells (79). However, although I_x1 is robust in experiments carried out using sharp microelectrodes on atrial (89) and ventricular (13) reaggregates, it has not been seen in whole cell clamp experiments carried out using patch pipettes on isolated 7- to 10-day ventricular cells (8) or on single 6- to 11-day atrial cells or small clusters of such cells (12). This might be due to an intrinsic absence of the current [e.g., there is good evidence for cell-cell contact-dependent regulation of expression of two different K+ channels in cultured adult rat ventricular cells (35)] or to rapid washout of this current in the whole cell ruptured-patch recording mode. Indeed, it has been suggested that the main difference in the action potentials of atrial reaggregates and small clusters of atrial cells can be accounted for by the absence of I_x1 in the latter (see Fig. 9 of Ref. 12 and Fig. 17 of Ref. 13). In contrast to the above-mentioned reports, in our 7-day ventricular clusters, the envelope-of-tails test shows two components (79), and application of the specific I_Kr blocker almokalant (113) removes the more rapidly deactivating component of the tail current. In addition, almokalant produces changes in the action potential consistent with I_Kr block (see below).

Fig. 2. Characteristics of slow delayed K+ current (I_Kr) in the model. A: steady-state activation (n) curve. B: time constant of activation (τ_n). C: fully activated (act) I_Kr. D: simulated current during voltage-clamp steps from a holding potential of −60 mV to −20, 0, and +20 mV (protocol of Ref. 8).

Temperature (Q10 ≈ 3 for peak current amplitude for guinea pig ventricular cells (79)). Figure 1D shows that the current traces from a voltage-clamp protocol in the model are similar, in magnitude and time course, to the corresponding experimental traces in Ref. 79 (see also Fig. 7B of Ref. 44, scaled for temperature and capacitance).

Because internal Ca2+ is buffered by our pipette solution, we do not include Ca2+-dependent inactivation of I_Kr (13) in our description; instead, we use only voltage-dependent inactivation to reproduce the time course of the inactivation process that is seen experimentally (44). Nor do we include the two time constants of inactivation seen in 7-day ventricular reaggregates (72).

Fig. 3. Characteristics of rapid delayed rectifier K+ current (I_Kr) in the model. A: steady-state activation (n) curve and rectification variable (z). B: time constant of activation (τ_n). C: fully activated I_Kr; note pronounced rectification. D: simulated current during voltage-clamp steps from a holding potential of −26 mV to −34, −43, −51, and −61 mV (protocol of Ref. 89).

As temperature (Q10 ≈ 3 for peak current amplitude for guinea pig ventricular cells (79)). Figure 1D shows that the current traces from a voltage-clamp protocol in the model are similar, in magnitude and time course, to the corresponding experimental traces in Ref. 79 (see also Fig. 7B of Ref. 44, scaled for temperature and capacitance).
89). Our form of \( z(V) \) (see APPENDIX; Fig. 3A) gives more current at depolarized voltages than the fit originally used in Fig. 8C of Ref. 89, which corresponds to the fact that we observe a maintained alkamolant-sensitive current at positive voltages (see RESULTS). The function \( z(V) \) represents the very rapid inactivation described for the human ether-a-go-go-related gene (HERG) subunit of \( I_{Kr} \) (92, 93) and for \( I_{Ko} \) in single SA node cells (54, 76). The reversal potential \( (E_{Ko}) \) was set to \(-81 \) mV on the basis of observations in our laboratory. (This closeness of \( E_{Ko} \) to the Nernst potential of \(-85 \) mV for \( K^+ \) is characteristic of \( I_{Ko} \) in many other cardiac preparations.) Our setting of the maximal conductance \( (g_{Ko}) \) yields a maximum value of the fully activated current (Fig. 3C) comparable to that seen in atrial reaggregates (89).

The gating variable \( s \) is governed by equations (see APPENDIX) slightly modified from those in Table I of Ref. 89, so as to fit more closely the data points in Fig. 5, A and B, of Ref. 89 (the original equations in Table I do not give the fitted curves shown in Fig. 5, A and B). With these modifications, our \( s_\infty \) and \( \tau_s \) curves (Fig. 3, A and B) are very close to the data in Fig. 5, A and B, of Ref. 89. A voltage-clamp protocol (Fig. 3D) gives currents similar to those in Fig. 4 of Ref. 89. We use only one time constant of activation; in SA node cells, two time constants of activation of \( I_{Ko} \) have been described (76).

\( I_{Ko} \).-\( V \)-curves of reaggregates of 7-day ventricular cells (14, 15), in small clusters of 7-day ventricular cells (79), and in single 7-day ventricular cells (4) show marked inward rectification at very negative potentials because of the presence of \( I_{Ko} \). There is also evidence for this current at the single-channel level in 7-day ventricular cells (67). We have thus included an \( I_{Ko} \) component in our model (Fig. 4), taking the formulation from a guinea pig ventricular cell model (65)

\[
I_{Ko} = g_{Ko}K1(V - E_{Ko})
\]

where we set \( E_{K1} = -81 \) mV, which is, as commonly observed, slightly depolarized to the calculated Nernst potential for \( K^+ \) (\(-85 \) mV). Although a time-independent description of \( I_{K1} \) (i.e., \( K_1 = K_{1\infty} \)) was originally used (65), we employ the time-dependent description to allow us to later formulate a model with stochastic gating kinetics to investigate beat-to-beat fluctuations in IBP (unpublished observations). However, the time constant of this current is so small \( (\tau_{K1} \leq 0.2 \) ms over the operative range of voltage; Fig. 4B) that the current is virtually identical in the time-dependent and time-independent descriptions. The maximal conductance \( (g_{K1}) \) is reduced from the guinea pig value to reflect the smaller \( I_{K1} \) earlier in development (15, 41). The steady-state \( I-V \) curve for the total current (see curve in Fig. 7A) is then very flat between \(-70 \) and \(-30 \) mV, which agrees with our experimental results (see symbols in Fig. 7A). The \( I_{K1} \)-\( V \)-curve (see Fig. 7C) is the main contributor to the positive slope of the total-current \( I-V \) curve at very hyperpolarized potentials (see Fig. 7A) and is similar to the \( Ba^{2+} \)-sensitive current at hyperpolarized potentials (4, 79).

\( I_{Na} \). In addition to \( I_{K1} \), which is outward at potentials depolarized to \(-81 \) mV, there is inward background current in 7-day ventricular reaggregates (14). This component has been modeled as a \( Na^+ \) current

\[
I_{Na} = g_{Na}(V - E_{Na})
\]

where \( E_{Na} = 40 \) mV and \( g_{Na} \) is obtained from Fig. 11 of Ref. 14, scaled for capacitance. Figure 7D gives the \( I-V \) relation for this linear current. \( I_{Na} \). It has been pointed out that \( I_{Na} \) flowing through the pipette gigaohm seal can be appreciable with respect to the total current flowing during diastolic depolarization for a very small cell with a high input resistance (20). We therefore add a nonspecific \( I_{Na} \) to our model

\[
I_{Na} = g_{Na}(V - E_{Na})
\]

with \( g_{Na} \) corresponding to a nominal seal-leak resistance of 5 G\( \Omega \) (20) and \( E_{Na} = 0 \) mV. Figure 7D gives the \( I-V \) relation of \( I_{Na} \).

**Currents Not Included in the Ionic Model**

\( I_{Na} \). There is voltage-clamp evidence for the existence of \( I_{Na} \) in reaggregates of 7- to 11-day ventricular cells (22, 72), in single 2- to 18-day ventricular cells (29, 40, 82, 83, 112), and at the single-channel level in 7-day ventricular cells (64, 112). Voltage-clamp studies of 7-day ventricular clusters in our laboratory show a fast inward current upon a depolarizing clamp step from potentials more hyperpolarized than about \(-60 \) mV. However, our clusters have a very low upstroke velocity (8.5 V/s), suggesting that \( I_{Na} \) might not contribute appreciably to the upstroke phase, especially because \( Ca^{2+} \) channel blockers abolish spontaneous activity (see Fig. 9, A and B). The MDP (\(-60 \) mV in the clusters and \(-67 \) mV in the model) is sufficiently depolarized to essentially render \( I_{Na} \) fully inactivated, because the foot of the \( I_{Na} \) steady-state inactivation curve lies at about \(-50 \) to \(-60 \) mV in 7-day ventricular reaggregates (22) and 7-day ventricular cells (29, 82). Indeed, addition of \( I_{Na} \) to our model, on the basis of the conductance and the activation and inactivation curves from single 7-day ventricular cells (29) and the time constants from 11-day reaggregates (22), slightly increases \( V_{max} \) from 9.5 to 10.2 V/s. In contrast, reaggregates of trypsin-dissociated 7-day ventricular cells have a TTX-sensitive upstroke velocity of 120 V/s in 1.3 mM \( K^+ \) and 91 V/s in 4.5 M \( K^+ \), presumably due to the more hyperpolarized MDP of about \(-90 \) and \(-76 \) mV, respectively (16, 19).

\( I_{Na} \) can also be involved in generating the pacemaker potential. There is indeed evidence that \( I_{Na} \) is necessary in some isolated embryonic cells for the generation of spontaneous activity. After 24 h in culture, 37% of single cells dissociated using trypsin from 7-day hearts (whole hearts, atria, or ventricles) stop beating after the addition of \( 10^{-5} \) g/ml TTX (70), showing that \( I_{Na} \) is crucial in generating spontaneous activity in these cells. However, the percentage of TTX-insensitive cells increases with time spent in culture: 4% at 4 h, 64% at 24 h, and 100% at 48 h (57). In contrast, reaggregates of trypsin-dissociated 7-day ventricular cells that are cultured for 24–72 h stop beating when exposed to TTX (16, 70). This difference in the response to TTX almost certainly indicates the importance of cell-to-cell interactions (19, 69). In newborn rabbit SA node, a TTX-sensitive current, which gradually disappears within the first 30 days postnatum, has been implicated in the generation of diastolic depolarization (2). This contribution is not due to the \( I_{Na} \) window current but, rather, is a consequence of relatively slow inactivation of \( I_{Na} \) in the pacemaker range of potentials. Recently, modeling work has suggested a role for a persistent component of a mutated \( I_{Na} \) in the generation of diastolic depolarization in long Q-T (LQT3) syndrome (105).

A third role for \( I_{Na} \) is maintenance of the plateau of the action potential, e.g., via a window current contribution. Application of TTX results in a shortening of the APD in some 7-day ventricular cells before they stop beating (64). Single \( Na^+ \) channels occasionally (1 of 100 beats) stay open throughout the action potential plateau (64) and burst for greater than 150 ms in 16% of trials during a long voltage-clamp step (40). Because these long openings do not persist into diastole (see Fig. 1 of Ref. 64), they would not contribute to diastolic depolarization. Incorporation of our standard Hodgkin-Huxley-type \( I_{Na} \) into the
model, as described above, results in a slight 3-ms increase in APD_{50} and a slight 5-ms increase in APD_{100}.

Pacemaker current. The pacemaker current (I_p) has been reported in ventricular reaggregates (4, 14, 15, 87, 88) as well as in single atrial and ventricular cells and small clusters of such cells (4, 5, 85). The midpoint of the activation range of I_p is ~30 mV more negative in single ventricular cells and small clusters than in reaggregates, with the foot of the activation curve of this hyperpolarization-activated current lying at ~70 mV in single cells and small clusters (5). In our clusters, we find I_p activated at potentials negative to ~70 mV (79). On the basis of the conductance, reversal potential, kinetics, and activation curve described in Ref. 5, we find that addition of I_p to the model causes only a very slight decrease in I_BI from 392 to 390 ms. The MDP in our cells is, hence, too depolarized for spontaneous beating 11-day reaggregates (100). In another report on clusters. In the SA node, the pacemaker current (I_p) has been reported in isolated ventricular cells possessing I_p increases with development, the number of outward current (I_O). For these reasons, we do not include it in our model.

Transient outward current. Although the size of the transient outward current (I_{trans}) increases with development, the number of isolated ventricular cells possessing I_{trans} is extremely low: 7 of ~300 cells at 3 days, 5 of ~200 cells at 10 days, and 5 of ~100 cells at 17 days (84). At the single-channel level, an outward channel appeared in only 1 of 80 patches from 7-day ventricular cells (67). I_{trans} was not seen in single atrial cells (12), nor was it "clearly observed" in 7- to 12-day atrial reaggregates (9). Moreover, in our own voltage-clamp experiments, we have also found no evidence of I_{trans} in the clusters. In the SA node, the I_{trans} density is smaller in cells with a smaller capacitance (58, 109). For these reasons, we do not include I_{trans} in our model.

Cl^- current. A time-independent Cl^- sensitive current (I_{Cl}) has been described in isolated 11-day ventricular cells (63). We do not include I_{Cl} explicitly in the model, but we consider it to be a component of I_O.

I_{NaK} and I_{NaCa}. Currents provided by ion pumps and exchangers, e.g., I_{NaK}, I_{NaCa}, and the Ca^{2+} pump, also contribute to V. We employ a first-generation model, which does not have an Na^-K^- pump, an Na^-Ca^2+ pump, internal Ca^{2+} dynamics, and variable ionic concentrations. However, I_{NaK} and I_{NaCa} are present in 7-day ventricular cells. Although these currents are included in several recent models of cardiac tissue, we do not include them in our model, because this would result in a second-generation model.

In our laboratory, 10 μM ouabain has been used to block I_{NaK} in 7- to 10-day ventricular cells or in small clusters of such cells (48). After 1 min of superfusion, the IBI first decreases, due to an increase in DDR, OS and MDP gradually fall, APD rises, and IBI increases, so that within a few minutes, spontaneous activity ceases, with the membrane coming to rest at about ~30 mV. (Sharp microelectrodes were used in these experiments, so that artifact due to dialysis and current rundown was minimal.) A similar result is seen in 11-day cells cultured as a confluent layer or polystrand, except the initial effect is seen immediately, presumably because of the use of a perfusion system with rapid perfusate changeover (half-time of ~5 s), with the membrane coming to rest at about ~40 mV (see Fig. 1 of Ref. 39). It has been estimated that I_{NaK} contributes 0.35 pA/pF at ~70 mV in spontaneously beating 11-day reaggregates (100). In another report on 11-day reaggregates at an internal [Na^+] of 41 mM, the ouabain-sensitive current amounts to 1.7 pA/pF and is independent of voltage over the operating range effective in our clusters (from ~60 to +20 mV) (see Fig. 1 of Ref. 99). This value scales to ~0.5 pA/pF at an internal [Na^+] of 10 mM (see Fig. 7 of Ref. 99), which agrees with SA node modeling work (54).

In our model, we can thus mimic the effect of blocking the electrogenic component of I_{NaK} by adding a constant depolarizing current of 10.2 pA (i.e., 0.4 pA/pF). This has the effect of decreasing IBI from 392 to 337 ms and depolarizing the MDP from ~67 to ~61 mV; both effects are seen immediately upon block of I_{NaK} in an experiment (see Fig. 18 of Ref. 39). Because the electrogenic component of I_{NaK} is removed within a few seconds of the start of block (Fig. 1 of Ref. 100), some secondary change must be responsible for the cessation of activity that occurs some minutes later (see Fig. 1 of Ref. 39). The most likely candidates are the rises in internal [Na^+] and [Ca^{2+}] after I_{NaK} block, which occur with a time course on the order of minutes (38, 39).

There is clear evidence for I_{NaCa} in 11-day ventricular reaggregates (100) and 11-day cells cultured as a confluent layer or polystrand (38). The maximum amplitude of I_{NaCa} in embryonic chick cells is about the same as in guinea pig ventricular cells (62). However, because of the difficulties inherent in interpreting experiments attempting to characterize I_{NaCa}, many of its fundamental properties (e.g., stoichiometry) remain uncertain in embryonic chick ventricular cells (62).

The extent to which I_{NaCa} is involved in generating diastolic depolarization in SA node cells is controversial (56) and is very different in different SA node models, to the extent that although I_{NaCa} is inward in most models, it is outward in at least one (see Fig. 7 of Ref. 54). In addition, in a model of spontaneous activity induced by suppression of I_{Cl} in guinea pig ventricular cells, I_{NaCa} is inward during the pacemaker potential (91). We are not aware of any studies of the Ca^{2+} pump in embryonic chick ventricular cells.

Given the above problems, as well as other problems described earlier involving degeneracy and drift in models where pumps and exchangers have been added, we have chosen not to include these currents in our model. Rather, I_{NaK} can be thought of as being incorporated into I_o, whereas the time course of I_{NaK} in our model very closely resembles the action potential clamp record (i.e., sum of I_{Ca} and I_{NaCa} and any Ca^{2+}-activated currents) obtained in the SA node when Ca^{2+} entry is blocked (120).

Other currents. Other currents, such as I_{Ia} [a sustained inward current, carried by Na^+, insensitive to TTX, and sensitive to Ca^{2+} channel blockers (71, but see Ref. 107)] and I_{K(Ca)} [a Ca^{2+}-activated K^- current, for which evidence is found only in the perforated-patch configuration (120)], exist in the SA node. Because there are no reports of these currents in ventricular cells, we do not include them in our model.

RESULTS

Spontaneous Activity

Action potentials recorded from 17 small clusters clearly show considerable cluster-to-cluster variability (Fig. 5) (17). Figure 6A shows a recording of V obtained from one small

![Transmembrane potential recorded during spontaneous activity from clusters 1-17. Tic marks indicate 0 mV.](http://ajpheart.physiology.org/10.1152/ajpheart.00314.2005)
cluster (cluster 7 in Fig. 5), whereas Fig. 6B shows the phase-plane trajectory, in which the rate of change of \( V \) (\( V' \)) is plotted vs. \( V \). For this cluster, the mean values of the parameters, averaged over 100 cycles of activity, were as follows: IBI = 458 ms, MDP = -57 mV, APA = 89 mV, \( V_{\text{max}} \) = 7.5 V/s, DDR = 85 mV/s, APD_{50} = 124 ms, and APD_{100} = 224 ms. The action potential parameters (means ± SD) of the 17 clusters are given in Table 1. Because of beat-to-beat variability, the action potential parameters for each cluster were averaged over \( \sim 100 \) beats before the population average was taken. Figure 6, C and D, gives the voltage-time series and the phase-plane trajectory for the ionic model, and Table 1 gives the action potential parameters in the model, which are quite close to the mean experimental values.

**Steady-State I-V Relations**

The curve in Fig. 7A gives the steady-state I-V relation for the total current in the model. This curve corresponds closely to the mean I-V data points obtained from five 7-day ventricular clusters in our laboratory (Fig. 7A, symbols); see also Ref. 4. The steady-state I-V relations of the individual currents in the model are shown in Fig. 7, B–D.

**Table 1. Action potential parameters: experiment vs. model**

<table>
<thead>
<tr>
<th></th>
<th>IBI, ms</th>
<th>( V_{\text{max}}, \text{V/s} )</th>
<th>MDP, mV</th>
<th>APA, mV</th>
<th>DDR, mV/s</th>
<th>APD_{50}, ms</th>
<th>APD_{100}, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>389 ± 78</td>
<td>8.5 ± 4.6</td>
<td>-60 ± 4</td>
<td>93 ± 7</td>
<td>110 ± 30</td>
<td>108 ± 24</td>
<td>176 ± 39</td>
</tr>
<tr>
<td>Model</td>
<td>392</td>
<td>9.5</td>
<td>-67</td>
<td>102</td>
<td>90</td>
<td>108</td>
<td>181</td>
</tr>
</tbody>
</table>

IBI, interbeat interval; \( V_{\text{max}} \), maximum rate of rise of upstroke; MDP, maximum diastolic potential; APA, action potential amplitude; DDR, diastolic depolarization rate; APD_{50} and APD_{100}, action potential duration at 50 and 100% repolarization. For each cluster, mean values of action potential parameters were computed for a train of \( \sim 100 \) action potentials. Experimental values are means ± SD of these mean values for 17 clusters.
Although 4 depolarization but gradually become less inward (Fig. 8, C and F), because the time constant for activation ($\tau_a$) is several hundred milliseconds over the pacemaker range of potentials (Fig. 3B). Hence, $I_{Kr}$ is not fully deactivated by the beginning of the upstroke of the action potential. However, the increase in voltage during the upstroke rapidly abolishes $I_{Kr}$ (Fig. 8C) because of its profound inward rectification (Figs. 3C and 8F). As the membrane then repolarizes, fast recovery from the inactivation of $I_{Kr}$ is responsible for its rectification (Fig. 8F). The time course of $I_{Kr}$ during spontaneous activity is very different from that seen in an atrial reaggregate model (see Fig. 15 of Ref. 89), where $I_{Kr}$ deactivates much more rapidly because of its shorter time constant at the more hyperpolarized MDP of the reaggregate model: about $-90$ mV (89) vs. $-67$ mV (present study). However, action potential clamp studies of rabbit SA cells, which are more depolarized than the chick atrial reaggregate, show a time course of $I_{Kr}$ very similar to that in our model (see Fig. 1C of Ref. 76), as do SA node models that incorporate a sharply rectifying $I_{Kr}$ component (see Fig. 6, D and E, of Ref. 54).

**Currents Underlying the Pacemaker Potential**

Because DDR in a three-cell cluster is $\sim 100$ mV/s (Table 1), the net current during diastolic depolarization is tiny ($\sim 2.6$ pA); it is not even appreciable on the scale of Fig. 8B. $I_{Ca}$ is inward and gradually becomes more inward throughout phase 4 depolarization (Fig. 8C), which agrees with the results from ruptured-patch action potential clamp studies on single SA node cells (120). $I_S$ and $I_{seal}$ are also inward throughout phase 4 depolarization but gradually become less inward (Fig. 8D). Although $I_{Ks}$, $I_{Kr}$, and $I_{K1}$ are outward during phase 4 depolarization, $I_{K1}$ becomes much less outward (Fig. 8D), $I_{Ks}$ gradually becomes slightly less outward (not visible on the scale of Fig. 8C) but does not contribute much current, and $I_{Kr}$ contributes increasingly less outward current as a result of slow deactivation (Fig. 8, C and F).

$I_{Kr}$ deactivates slowly during diastolic depolarization (Fig. 8, C and F), because the time constant for activation ($\tau_a$) is several hundred milliseconds over the pacemaker range of potentials (Fig. 3B). Hence, $I_{Kr}$ is not fully deactivated by the beginning of the upstroke of the action potential. However, the increase in voltage during the upstroke rapidly abolishes $I_{Kr}$ (Fig. 8C) because of its profound inward rectification (Figs. 3C and 8F). As the membrane then repolarizes, fast recovery from the inactivation of $I_{Kr}$ is responsible for its rectification (Fig. 8F).

**Effect of Ca$^{2+}$ Channel Blockers on Spontaneous Activity**

Application of D-600, a Ca$^{2+}$ channel blocker, on 7- to 10-day cells and small clusters in our laboratory results in the abolition of spontaneous activity (Fig. 9A), with mean resting potential of $-36.2$ mV ($n = 14$) (49). We observed similar results with another Ca$^{2+}$ channel blocker, diltiazem (51). In Fig. 9A, a sharp microelectrode is used so that the cessation of spontaneous activity is not due to dialysis of the pipette contents against the intracellular medium, leading to effects such as current rundown.

Gradually increasing block of $I_{Ca}$ in the model, starting at 45 s in Fig. 9C, gives a time course of the voltage that is similar to the experimentally observed effect of D-600, with the membrane eventually coming to rest at $-37$ mV at $\sim 120$ s when $\sim 90\%$ of $I_{Ca}$ is blocked. Because D-600 blocks $I_{Ca,L}$ and because our nonseparable $I_{Ca}$ is close to $I_{Ca,L}$, the modeling intervention is similar to the experimental intervention of applying an $I_{Ca,L}$ blocker. In the experiment and the model, loss of OS initially proceeds at a slow rate (from just after arrow 1 to just after arrow 3 in Fig. 9C); then the rate of loss accelerates just before spontaneous activity is extinguished (i.e., just after arrow 3 in Fig. 9C). The MDPI initially drifts slowly positive and then suddenly depolarizes much more quickly (starting at arrow 2 in Fig. 9C) before spontaneous activity ceases. In the experiment and the model, the phase of more rapid loss of MDPI precedes the phase of more rapid loss of OS. The upstroke velocity gradually decreases throughout the course of the block, and APD$_{100}$ increases (Fig. 9, B and D). Effects in many ways opposite to those described above are seen in our laboratory with administration of a Ca$^{2+}$ channel agonist (BAY K 8644): there are increases in $V_{max}$, OS, DDR, and APD, as well as a hyperpolarization of MDPI and the threshold or take-off potential, and a fall in IBI (28); these changes are also seen in the model.

Figure 9E gives the bifurcation diagram for $I_{Ca}$ block, computed using XPPAUT (25). The bifurcation parameter is $g_{Ca}$, and the bifurcation variable is $V$. The periodic activity of

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**Fig. 8.** Spontaneous activity in the model. A: transmembrane potential. B: total current. C and D: individual currents. Peak value of $I_{Ca}$ is $-262$ pA (off-scale deflection in C). E and F: activation and inactivation variables in the model. Also shown is the function $z(V)$, describing rectification of $I_{Kr}$.
The resting membrane potential corresponding to this stable steady state produced in the subcritical Hopf bifurcation. At the control value of 30 nS (Fig. 9C), the more rapid phase of decline of MDP starts earlier, before the falling off (just after arrow 2), at \( g_{\text{Ca}} \approx 6 \) nS, which agrees with the change in the slope of MDP in the bifurcation diagram (Fig. 9E). [This pattern is also seen in the experiment (Fig. 9A).]

When a trace such as that shown in Fig. 9C, with distinct phases of change of MDP and OS, is seen experimentally, one should begin to think that abolition of spontaneous activity might involve a subcritical, rather than a supercritical, Hopf bifurcation.

The existence of a saddle-node bifurcation in Fig. 9E is consistent with three prior observations from our laboratory: 1) During washout of D-600, transient flurries of action potentials occur spontaneously before spontaneous activity is permanently reestablished. The amplitude of the first action potential in each flurry is relatively large, with the amplitude of the following action potentials gradually decreasing during the course of each episode of transient triggered activity (see Fig. 2C of Ref. 49). 2) Once beating has stopped under the influence of diltiazem, injection of a hyperpolarizing bias current can provoke an episode of transient triggered activity, with the first action potential being an anodal-break response (see Fig. 2 of Ref. 51). As time proceeds and the degree of block continues to increase during quiescence, the number of action potentials in an episode decreases. This “critical slowing-down” behavior is consistent with the existence of a saddle-node bifurcation of limit cycles and can be seen in simulations with the model. 3) In some cells that are initially found to be not spontaneously active, injection of a single hyperpolarizing current pulse again elicits a flurry of triggered action potentials, with the action potential amplitude gradually decreasing during each flurry (see Figs. 4 and 6 of Ref. 49). During ongoing superfusion of these cells with D-600, the number of nondriven action potentials in any one trial gradually decreases from tens of action potentials to a few, with the number of nondriven action potentials gradually decreasing during each flurry.
occurs, so that annihilation and single-pulse triggering cannot occur.

Effect of Almokalant on Spontaneous Activity

Addition of 1 or 2 mM almokalant, a specific blocker of $I_{Kr}$ (113), to the bath results in a slowing of the terminal rate of repolarization, a small depolarization of the MDP, and a slight loss of OS (Fig. 10A).

In the model, 100% block of $I_{Kr}$ (Fig. 10B) shows 50%, as well as 100%, block of $I_{Kr}$ results in a marked depolarization of MDP, a slight slowing of the terminal rate of repolarization, a small increase in $APD_{100}$, a decrease in $APD_{50}$, a decrease in IBI, a fall in $V_{\text{max}}$, and a decrease in OS. The slowing of the terminal rate of repolarization and the depolarization of the MDP are due to the absence of the $I_{Kr}$ contribution normally present (see $I_{Kr}$ trace in Fig. 8C during control activity). The resultant relative depolarization then causes $I_{Kr}$, to remain considerably more outward during the entire pacemaker potential and even during the early part of the action potential (Fig. 10C shows $I_{Kr}$, time course during 100% $I_{Kr}$ block), which is the major cause of the decrease in $APD_{50}$ in Fig. 10B. This secondary increase in $I_{Kr}$ also accounts for the relatively small effect of the loss of $I_{Kr}$ on the rate of terminal repolarization in Fig. 10B. In cells that do not have $I_{Kr}$, this indirect effect of $I_{Kr}$ block on $I_{Kr}$ would not occur, and one would expect a prolongation of APD, as observed in SA node cells in which $I_{Kr}$ was not found (106). [In later studies, however, $I_{Kr}$ was clearly found in SA node cells (59, 110).] The fall in $V_{\text{max}}$ and OS is due to a decrease in $I_{Ca_{\text{w}}}$, with a fall in peak value from 263 to 207 pA during 100% $I_{Kr}$ block as a result of greater inactivation of $I_{Ca_{\text{w}}}$ during diastolic depolarization. Similarly, in experiments on the SA node, effects on action potential parameters due to selective block of $I_{Kr}$ with the compound E-4031 have been found to be due to “a combination of direct and indirect effects on various ionic currents” (106).

The shortening of $APD_{50}$ in the model with $I_{Kr}$ block (Fig. 10B) does not occur in the experiment (Fig. 10A). One reason that might account for this discrepancy is the rundown of $I_{Kr}$ that occurs during the 10-min period between rupture of the patch (to enter whole cell recording mode) and initiation of the recording of the effect of almokalant (79). Figure 10D shows the combined effect in the model of 50% block of $I_{Kr}$ (to simulate almokalant) and 20% block of $I_{Kr}$ [to simulate the degree of rundown seen experimentally (79)]: $APD_{50}$ is no longer decreased.

To avoid the above-mentioned artifact due to dialysis of cytoplasm against the pipette contents and rundown of currents, we also used visual monitoring of mechanical beating to assess the effect of almokalant. Although three clusters stopped beating on exposure to almokalant (and the effect was reversible on washout of almokalant from the bath), another four clusters did not stop beating: IBI was increased in three of four clusters, and there was no change in the remaining cluster. In response to $0.1 \mu M$ E-4031, another specific $I_{Kr}$ blocker, half of the single SA node cells stopped beating in one study (106), whereas none stopped in another study (76). When the concentration was raised to $1.0 \mu M$, at which there are still negligible effects on other currents, all cells ceased spontaneous activity in both studies. A similar response was found in small balls of tissue isolated from the SA node: with $1.0 \mu M$ E-4031, activity was abolished in all balls; with $0.1 \mu M$ E-4031, activity was sometimes extinguished in balls from the central area, but not in those from more peripheral areas (46). The fact that $0.1 \mu M$ E-4031 abolishes spontaneous activity in smaller, but not in larger, single SA node cells agrees with this observation (59), provided that small cells do indeed stem from the central area of the node and larger cells stem from the periphery.

Complete block of $I_{Kr}$ does not abolish spontaneous activity in the model (Fig. 10B). In contrast, cessation of spontaneous activity does occur with complete $I_{Kr}$ block in some SA node models, sometimes via a supercritical Hopf bifurcation, sometimes via what is probably a subcritical Hopf bifurcation, and sometimes through an intermediary phase of “irregular dynamics” (see Fig. 13 of Ref. 54).

Effect of $Ba^{2+}$ on Spontaneous Activity

In 7-day ventricular cells, a low concentration of $Ba^{2+}$ (0.2 mM) blocks $I_{K1}$ without greatly affecting $I_{Kr}$ and $I_{Ca}$ (8). Figure 11A shows the effect of $0.1 \mu M$ $Ba^{2+}$ in an experiment, and Fig. 11B shows the effect of blocking $I_{Kr}$ completely in the model. In the experiment and the model, there is a depolarization of MDP, a decrease in $V_{\text{max}}$, a loss of OS, and a decrease in IBI. With the average drift of action potential parameters in control recordings after 10 min (the time after which the effects due to $Ba^{2+}$ were measured) taken into account, MDP depolarizes by 5%, $V_{\text{max}}$ decreases by 13%, APA decreases by 7%; and IBI decreases by 9% ($n = 4$) (79), whereas in the model, MDP depolarizes by 15%, $V_{\text{max}}$ decreases by 28%, APA decreases by 13%, and IBI decreases by 11%. However, the increases in APD and DDR in the experiments (12% increase in $APD_{50}$ and 28% increase in $APD_{90}$) are not replicated in the model. Because we have observed neither an increase in the peak $I_{Ca}$ nor slowed inactivation kinetics of $I_{Ca}$ with elevated $Ba^{2+}$ (79), we attribute the increase in APD in the experiments

![Image](http://ajpheart.physiology.org/DownloadedFrom/26980680.png)
at least partially to rundown of $I_{Ks}$, as we observed with almokalant (see Effect of Almokalant on Spontaneous Activity).

Increasing $\text{Ba}^{2+}$ to 0.5–1.0 mM results in partial block of $I_{Ks}$ (8, 79) and $I_{Kr}$ (79), in addition to complete block of $I_{K1}$. Figure 11C shows the mean effect ($n = 9$) on the steady-state $I-V$ relation when 0.5 mM $\text{Ba}^{2+}$ is applied to small clusters. Figure 11D shows the effect on the steady-state $I-V$ relation in the model of 100% block of $I_{K1}$, 33% block of $I_{Ks}$, and 33% block of $I_{Kr}$. (The degree of block of $I_{Ks}$ and $I_{Kr}$ in the model was chosen to make the blocked current comparable with the combined effects of rundown and $\text{Ba}^{2+}$ in Fig. 11C). The steady-state $I-V$ curve is no longer N-shaped. The effect on spontaneous activity in the experiment and the model is a more marked change from control in the action potential parameters than at the lower $\text{Ba}^{2+}$ concentration (Fig. 11, A and B).

Specifically, in the experiment, again with the average drift over a period of 10 min in action potential parameters in control recordings taken into account, the MDP depolarizes by 27%, $V\text{max}$ decreases by 49%, APA decreases by 15%, and IBI decreases by 34% ($n = 8$) (79), whereas in the model, MDP depolarizes by 27%, $V\text{max}$ decreases by 39%, APA decreases by 23%, and IBI decreases by 20%.

At an even higher concentration (1 mM), $\text{Ba}^{2+}$ abolishes spontaneous activity in the clusters, with the membrane coming to rest at about $-35$ mV (79). The effect is reversible, in that spontaneous activity resumes with washout of $\text{Ba}^{2+}$. Depolarization of MDP and cessation of spontaneous activity have previously been described in 6- to 7-day ventricular cells exposed to 5–10 mM $\text{Ba}^{2+}$ (96). Cessation of spontaneous activity is also seen in the model if, in addition to complete block of $I_{K1}$, $I_{Ks}$, and $I_{Kr}$ are blocked by 68%, with the membrane coming to rest at $-17$ mV. In this case, the activity is abolished through a supercritical Hopf bifurcation. This modeling result agrees with there being only one distinct phase of change of MDP and OS in the experiment (79), in contrast to the result of $I_{Ca}$ block (Fig. 9). Blocking the other background current in the model ($I_{B}$) results in the cessation of spontaneous activity via an infinite-period bifurcation.

Effect of $I_{\text{seal}}$ on Spontaneous Activity

It is possible that $I_{\text{seal}}$ is essential for the generation of spontaneous activity in our model. However, when $I_{\text{seal}}$ is removed, spontaneous beating continues in the model, although at a considerably increased IBI of 535 ms and a more hyperpolarized MDP of $-72$ mV (Fig. 12). This result agrees with the fact that we record from clusters that are seen to be beating before they are subjected to patching; i.e., the depolarizing $I_{\text{seal}}$ does not induce spontaneous beating in our clusters. However, patching onto a single cell can change the IBI or even abolish preexisting spontaneous activity (112). Indeed, if our model (with the nominal seal-leak resistance of 5 GΩ) is reformulated for a single cell (i.e., capacitance = 8.5 pF), the MDP depolarizes to $-51$ mV and the IBI decreases to 293 ms; with a seal-leak resistance of 3 GΩ, spontaneous activity is abolished in the single-cell model.

We have used a nominal seal-leak resistance of 5 GΩ in our model (20). This is a worst-case scenario, because 5 GΩ is toward the lower end of the range of values reported in experimental work on chick cells (42, 63, 79). When the seal-leak resistance in the model is increased to 20 GΩ, which is toward the higher end of that used in our laboratory (79), IBI increases from 392 to 475 ms. Thus differences in seal-leak resistance might account for part of the prepara-

**Fig. 12. Effect of removal of $I_{\text{seal}}$ in the model.**
tion-to-preparation differences in IBI and DDR seen experimentally (Fig. 5).

DISCUSSION

Spontaneous Activity in Isolated Chick Ventricular Cells

Electrical recordings of spontaneous activity in single embryonic chick ventricular cells, in small clusters of such cells, and in sparse monolayers have been made using conventional sharp microelectrodes (17, 26, 48, 49, 51, 78, 95) or patch pipettes in the whole cell recording mode (64, 67, 112). Among these studies, those that use 7-day ventricular cells at an external [K+] of 2.7–5.4 mM generally report that the cells have an MDP between −60 and −70 mV and a slow upstroke velocity of ~10 V/s. One systematic study, which used sharp microelectrodes, reported MDP of −68.4 ± 1.0 mV and OS of 22 ± 0.9 mV (n = 30) at an external [K+] of 4.2 mM (17). Our findings in small clusters (Table 1) are in agreement with the findings of these previous studies.

How does the electrical activity of small clusters compare with that of the in situ ventricular muscle? The APA and APD of our small clusters (and in the other reports on single cells, small clusters, and sparse monolayers mentioned above) are quite similar to those of in situ ventricular muscle (98, 101, 118). In contrast, in situ ventricular muscle, with one exception (97), has not been reported to show spontaneous phase 4 depolarization (61, 101, 118) vs. 8.5 V/s (present study). The absence of diastolic depolarization in the in situ 7-day chick ventricle could be due to overdrive suppression, because, in the only report in which pacemaker potentials were seen in intact 7-day ventricles, such potentials were seen in only 20–40% of intact ventricles but in 100% of cut 7-day ventricular fragments (61, 101, 118) and has a much larger V_max: 70–94 V/s at 5–7 days (19, 98, 118) vs. 8.5 V/s (present study). For example, in the smallest SA node cells (presumably from the central part of the node), which have a capacitance about 20 pF), the Iseal has seal-leak resistance results in the cessation of spontaneous activity.

Very early during development (3 days), the steady-state inactivation curve of I_Na is shifted in the depolarizing direction (82); simulations suggest that the window component of I_Na might then contribute to diastolic depolarization (83). I_Na has been reported during diastolic depolarization in spontaneously active single SA node cells (71, but see Ref. 107); intriguingly, I_Na is not present in quiescent SA node cells (71). We know of no reports of I_Na in embryonic chick cells.

I_seal injected through the gigahm seal-leak resistance is a source of artifact in our experimental recordings. This is true even if the perforated-patch, rather than the ruptured-patch, technique were to be used. In contrast to the case in relatively large adult mammalian ventricular cells with input resistances on the order of tens of megohms, I_seal has significant effects when smaller cells (e.g., SA node cells and embryonic cells) with membrane resistances on the order of 1 GΩ at ~70 mV [as do our clusters; see also Ref. 29] are studied (20). It is thus conceivable that I_seal is causing depolarization to the point that I_Na and I_Ca,T are blocked and I_f becomes deactivated in SA node cells. However, in our model, subtraction of I_seal hyperpolarizes the MDP by only 6 mV (resp 2 mV) while increasing the IBI from 392 to 535 ms (resp. 472 ms) when the seal-leak resistance is 5 GΩ (resp. 20 GΩ). This extra hyperpolarization is not sufficient to activate significant amounts of I_Na, because when I_Na is added to the model (see METHODS), removal of the 5-GΩ seal-leak resistance results in a decrease in IBI from 392 to 533 ms and an increase in V_max from 10.2 to 10.9 V/s. Similarly, when I_f is added to

Currents Underlying Diastolic Depolarization in the Model

A DDR of ~100 mV/s (Table 1) in a three-cell cluster with a capacitance of 25.5 pF requires that the net current flowing during spontaneous diastolic depolarization be 2.55 pA. In our model, this net current is obtained from the algebraic sum of several much larger inward and outward currents (Fig. 8). In models of SA node cells, these individual currents contribute to various extents to produce this tiny net current (see Fig. 4 of Ref. 115 and Figs. 6 and 7 of Ref. 54). At present, we have no way of knowing exactly which currents are flowing and in what amounts during diastolic depolarization in a given cell; to make matters worse, this mix of currents almost certainly changes from cell to cell, especially in cells from structures as inhomogeneous as the SA node (47).

The current for which we have the least direct experimental evidence in our model is I_st (14). We have added this current to the model, because we know that an inward background current is needed to generate a steady-state I-V curve that agrees with the experiment (Fig. 7A) and to allow the membrane to come to rest at about −40 mV when I_Ca is blocked (Fig. 9). Background currents have also been described in SA node cells (34, 106). One or more inward background currents are incorporated into all models of atrial (9, 52, 88–90) and ventricular (11, 88) reaggregates, as well as into all models of SA node cells (see Fig. 4 of Ref. 115 and Fig. 7 of Ref. 54). As in many of the above-mentioned models, the inward background current is necessary for spontaneous activity in our model, in that deletion of the inward background current results in the cessation of spontaneous activity.

For example, in the three-cell clusters (i.e., ~20 pF), currents such as I_Ca,L, I_Ks, and I_Ks and background currents are present, but other currents such as I_Na, I_f, and I_Ca,T are absent or the membrane is too depolarized to allow activation (e.g., I_f) or to allow removal of inactivation (e.g., I_Na) (36). Indeed, a minimal SA node model with only I_Ca, I_Ks, and a background current produces very respectable-looking spontaneous activity (33).
the model (see METHODS), the extra hyperpolarization does not activate \( I_L \) significantly: in this case, removal of \( I_{seal} \) increases the IBI from 390 to 520 ms.

**Spontaneous Activity: Cell-to-Cell Variability and Activity-Dependent Conductances**

There is considerable variability in electrical parameters from cluster to cluster (Fig. 5). The extent to which this is a result of true inhomogeneity within the cellular population [e.g., the regional heterogeneities in the early embryonic chick heart (1, 101) and the apex-to-base and endocardial-to-epicardial ventricular gradients known to be present in adult muscle (77)] is not clear. There is almost certainly an artifact stemming from differences in dissociation conditions from one culture to another (and even from cluster to cluster within the same culture). \( I_{seal} \) is also different from cluster to cluster. Population inhomogeneity exists in ventricular cells isolated from the adult rat heart (77) and the adult guinea pig heart (119), as well as in cells isolated from the rabbit SA node (75). Although at first sight these cell-to-cell differences might appear to be so large as to be problematic for physiological function, experimental and modeling work indicates that cell-to-cell differences in APD would be largely smoothed out in situ by gap-junctional coupling (119); a similar conclusion holds for IBI (108).

The action potential parameters in the model are very close to the mean values obtained in the experiment (Table 1). However, because of the inhomogeneity of the cellular population, a particular action potential parameter in a given cluster can be quite different from that in the model. For example, in Fig. 5 the MDP in the model is more depolarized than in cluster 16, the IBI is much smaller than in cluster 17, and the APD is much shorter than in cluster 2. In each of these cases, the model could presumably be modified to replicate the electrical activity in the particular cluster by changing particular currents (e.g., increasing the conductance of one or more of the \( K^+ \) currents to produce a more hyperpolarized MDP or increasing the conductance of \( I_{Ca} \) to extend the APD). However, this is an exercise in “theorizing in a vacuum,” given that one does not know (and cannot know, with the available technology) the exact parameters describing all the ionic currents in a given cell.

The wide variety of shapes of the action potentials seen in the experiment and the corresponding cell-to-cell variability in the individual currents must mean that the currents influencing APD and DDR (and, hence, IBI) are mixed to various extents in different clusters. Because ~40% of our clusters do not beat (50), it is perhaps not surprising that there is a great deal of cluster-to-cluster variability in IBI and in the waveform of the action potential. It has been suggested that activity-dependent conductances might provide a negative-feedback mechanism by which spontaneous activity can be regulated (for review see Ref. 80). It is quite conceivable that some such homeostatic process is occurring here, because, e.g., it is known that messenger RNA expression and protein level of one \( K^+ \) channel are decreased as a result of KCl-induced depolarization in pituitary cells (60). It is also possible that the individual currents, through their voltage dependence, negatively feed back on each other to regulate spontaneous activity (73).

**Limitations of the Model**

Our first-generation model contains no pumps or exchangers and has fixed ionic concentrations. It thus cannot be used to realistically model phenomena such as the rise in internal \([Na^+]_i\) seen when \( I_{Na,K} \) is blocked or the change in internal \([Ca^{2+}]_i\) that occurs should IBI be changed. Because data are not available for all the currents in 7-day embryonic chick ventricular cells, there are uncertainties in some of the currents: \( I_{K1} \) is taken from experiments on atrial reaggregates, and we use a nonspecific \( I_{Ca} \). Although we have used the classic Hodgkin-Huxley description for the gating of all the currents in our model, there are channels that do not always behave in this way, e.g., the “bursting” mode of \( I_{Na,K} \), which occasionally results in a longer-lasting current (40, 64). As in all work on ionic models of spontaneously active cells, \( I_h \), which in our case is a composite current (including, e.g., \( I_{Na,K} \)), is titrated to produce a reasonable IBI. There is evidence from recent work on the SA node that internal \([Ca^{2+}]_i\) cycling contributes to generating spontaneous diastolic depolarization (56). Because there is no firm experimental evidence for this mechanism in embryonic chick ventricular cells, we do not entertain this possibility in our model.

The deterministic Hodgkin-Huxley-type model we present here shows no beat-to-beat fluctuation in action potential parameters. In contrast, all of our experimental recordings show considerable beat-to-beat fluctuations in the various action potential parameters (e.g., IBI). Previous modeling studies of rabbit SA node cells showed that the beat-to-beat fluctuations observed experimentally can be accounted for by the stochastic opening and closing of membrane channels (32, 114). Development of a stochastic single-channel version of the deterministic model we have described above allows investigation of this hypothesis (unpublished observations).

**APPENDIX**

**Model Equations**

Some model parameters (maximal conductances, reversal potentials, and capacitance) are given in Table 2.

\[
dV/dr = -(I_{Ca} + I_{K1} + I_{Ks} + I_{Kr} + I_h + I_{seal})/C_m
\]

**Table 2. Model parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g_{Ca} )</td>
<td>Maximal ( I_{Ca} ) conductance</td>
<td>30 nS</td>
</tr>
<tr>
<td>( g_{K1} )</td>
<td>Maximal ( I_{K1} ) conductance</td>
<td>7.8 nS</td>
</tr>
<tr>
<td>( g_{Ks} )</td>
<td>Maximal ( I_{Ks} ) conductance</td>
<td>6.0 nS</td>
</tr>
<tr>
<td>( g_{Kr} )</td>
<td>Maximal ( I_{Kr} ) conductance</td>
<td>3.6 nS</td>
</tr>
<tr>
<td>( g_b )</td>
<td>Maximal ( I_b ) conductance</td>
<td>0.2 nS</td>
</tr>
<tr>
<td>( g_{seal} )</td>
<td>Maximal ( I_{seal} ) conductance</td>
<td>0.2 nS</td>
</tr>
<tr>
<td>( E_{Ca} )</td>
<td>Reversal potential of ( I_{Ca} )</td>
<td>40 mV</td>
</tr>
<tr>
<td>( E_{K1} )</td>
<td>Reversal potential of ( I_{K1} )</td>
<td>-75 mV</td>
</tr>
<tr>
<td>( E_{Ks} )</td>
<td>Reversal potential of ( I_{Ks} )</td>
<td>-81 mV</td>
</tr>
<tr>
<td>( E_{Kr} )</td>
<td>Reversal potential of ( I_{Kr} )</td>
<td>-81 mV</td>
</tr>
<tr>
<td>( E_b )</td>
<td>Reversal potential of ( I_b )</td>
<td>40 mV</td>
</tr>
<tr>
<td>( E_{seal} )</td>
<td>Reversal potential of ( I_{seal} )</td>
<td>0 mV</td>
</tr>
<tr>
<td>( C_m )</td>
<td>Membrane capacitance</td>
<td>25.5 pF</td>
</tr>
</tbody>
</table>

\( I_{Ca} \), \( Ca^{2+} \) current; \( I_{K1} \), slow delayed \( K^+ \) current; \( I_{Ks} \), rapid delayed rectifier \( K^+ \) current; \( I_{Kr} \), inward rectifier \( K^+ \) current; \( I_h \), background current; \( I_{seal} \), seal-leak current.
**Slow Inward \( Ca^{2+} \) Current**

\[
I_{C_{a}} = g_{C_{a}}d(V - E_{C_{a}})
\]

\[
d(t) = \frac{1}{\tau_{a}}(d_{a} - d)
\]

\[
d(t) = \frac{1}{\tau_{f}}(d_{f} - f)
\]

\[
f_{a} = \frac{1}{1 + \exp[(V + 42.8)/8.4]}\]

\[
d_{a} = \frac{1}{1 + \exp[-(V + 10.0)/6.2]}
\]

\[
\tau_{d} = 0.001 \times (1 - \exp[-(V + 10.0)/6.24]/(1 + \exp[-(V + 10.0)/6.24]) \times (0.035(V + 10))
\]

\[
\tau_{f} = 0.001/(0.0197exp(-0.0337(V + 10)^2) + 0.02)
\]

**Slow Delayed K\(^+\) Current**

\[
I_{k_{s}} = g_{k_{s}}z(V - E_{k_{s}})
\]

\[
z(V) = \frac{1}{1 + \exp[((V + 100)/25)]}
\]

\[
d(s) = \frac{1}{\alpha_{s}(1 - s) - \beta_{s}z}
\]

\[
\alpha_{s} = 23.0exp(0.13(V + 9.0))\]

\[
\beta_{s} = 0.036exp(-0.09(V + 9.0))
\]

**Rapid Delayed Rectifier K\(^+\) Current**

\[
I_{k_{1}} = g_{k_{1}}K_{1}(V - E_{k_{1}})
\]

\[
d(K_{1}) = \frac{1}{\alpha_{k_{1}}(1 - K_{1}) - \beta_{k_{1}}K_{1}}
\]

\[
\alpha_{k_{1}} = 1.000 \times 1.02/[1 + \exp(0.2385(V - E_{k_{1}} - 59.215))]
\]

\[
\beta_{k_{1}} = 1.000 \times (0.49124exp(0.08032(V - E_{k_{1}} + 5.476)]
\]

\[
+ \exp(0.06179(V - E_{k_{1}} - 59.431))/\]

\[
1 + \exp[-0.5143(V - E_{k_{1}} + 4.753))]
\]

**Inward Rectifier K\(^+\) Current**

\[
I_{k_{1}} = g_{k_{1}}K_{1}(V - E_{k_{1}})
\]

\[
d(K_{1}) = \frac{1}{\alpha_{k_{1}}(1 - K_{1}) - \beta_{k_{1}}K_{1}}
\]

\[
\alpha_{k_{1}} = 1.000 \times 1.02/[1 + \exp(0.2385(V - E_{k_{1}} - 59.215))]
\]

\[
\beta_{k_{1}} = 1.000 \times (0.49124exp(0.08032(V - E_{k_{1}} + 5.476)]
\]

\[
+ \exp(0.06179(V - E_{k_{1}} - 59.431))/\]

\[
1 + \exp[-0.5143(V - E_{k_{1}} + 4.753))]
\]

**Background Current**

\[
I_{b} = g_{b}(V - E_{b})
\]

**Seal-Leak Current**

\[
I_{\text{seal}} = g_{\text{seal}}(V - E_{\text{seal}})
\]

Voltagess are in millivolts, currents in picoamperes, conductances in nanosiemens, capacitance in nanofarads, and time in seconds. The initial conditions are \( V = -66.5526 \text{ mV}, d = 1.0932 \times 10^{-4}, f = 0.6309, n = 0.0764, s = 0.8019, \) and \( K_{1} = 0.2953. \)

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**REFERENCES**


89. Shrier A and Clay JR.
90. Silva J and Rudy Y.
91. Shrier A and Clay JR, and Brochu RM.
92. Sada H, Ban T, Fujita T, Ebina Y, and Sperelakis N.
93. Sachs HG, McDonald TF, and DeHaan RL.
94. Satoh H.
95. Schanne OF, Qu J, Haddad GE, and Ruiz-Petrich E.
97. Smith PL, Baukrowitz T, and Yellen G.
98. Sperelakis N and Shigenobu K.
99. Stimers JR, Liu S, and Lieberman M.
100. Veldkamp MW, de Jonge B, and van Ginneken ACG.


80. Sada H, Ban T, Fujita T, Ebina Y, and Sperelakis N.
81. Sachs HG, McDonald TF, and DeHaan RL.
82. Satoh H, Ban T, Fujita T, Ebina Y, and Sperelakis N.
83. Schanne OF, Qu J, Haddad GE, and Ruiz-Petrich E.
84. Satoh H. Identification of and developmental changes in transient outward current in embryonic chick cardiomyocytes.
85. Satoh H and Sperelakis N.
86. Schanne OF, Qu J, Haddad GE, and Ruiz-Petrich E.
87. Shrier A and Clay JR.
88. Smith PL, Baukrowitz T, and Yellen G.
89. Shrier A and Clay JR.
90. Silva J and Rudy Y.
91. Smith PL, Baukrowitz T, and Yellen G.
92. Spector PS, Curran ME, Zou A, Keating MT, and Sanguinetti MC.
93. Sperelakis N. Electrical properties of embryonic heart cells.
94. Sperelakis N and Lehmkulh D.
95. Sperelakis N and Lehmkulh D.
96. Sperelakis N and McLean MJ.
97. Shrier A and Clay JR.
98. Sperelakis N and Shigenobu K.
99. Stimers JR, Liu S, and Lieberman M.
100. Veldkamp MW, de Jonge B, and van Ginneken ACG.


