Overdrive suppression of spontaneously beating chick heart cell aggregates: experiment and theory

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Recently, we characterized overdrive suppression in spontaneously beating chick heart cell aggregates (30). We studied the kinetics of buildup and decay of the overdrive suppression after electrical stimulation at constant frequency and demonstrated the role of overdrive suppression in the evolution of rhythms during periodic stimulation. Here, we extend this work by considering the effects of stimulation frequency, amplitude, and duration of pacing on overdrive suppression. Although we are interested in the detailed ionic mechanisms of the heart cell aggregates (14), we believe that development of simplified theoretical models can complement the ionic models by providing easily understandable equations demonstrating the main phenomena. We propose a system of nonlinear ordinary differential equations to model the cardiac oscillator on the basis of the van der Pol equation (6, 23). This oscillator equation is modified by implementation of an additional equation to account for overdrive suppression on the basis of the hypothesis that rapid stimulation induces an electrogenic outward current (24). We assume that each action potential induces an outward current that decays slowly during diastole. At rapid stimulation rates, there is inadequate time between action potentials for the outward current to return to control levels, leading to an increased outward current and lower spontaneous frequency. The outward current plays a role during control activity as well as during electrical stimulation. The experimental results concerning the buildup and decay of overdrive suppression are in good agreement with the simulations of the theoretical model.

MATERIALS AND METHODS

Tissue Culture

Aggregates were prepared using techniques described previously (5, 12). White Leghorn chick embryos were incubated for 7 days at 37°C and 85% relative humidity. They were then decapitated, and their hearts were excised. Atria and ventricles were isolated, fragmented, and then dissociated into single cells in a deoxyribonuclease- and trypsin-containing medium (5). The resulting cell suspension was filtered through a 12.0-μm-diameter-pore-size filter and centrifuged for 15 min at 170 g. The cells were resuspended and aliquoted into 25-ml Erlenmeyer flasks containing 3 ml of maintenance medium at densities of 5 × 10^6-7 × 10^6 cells/flask. The flasks were then gassed with 5% CO_2-10% O_2-85% N_2, sealed with a silicone rubber stopper, and placed on a gyratory table (70 rpm and 37°C) to allow the formation of spherical aggregates.

The dissociation medium contained 5.25 × 10^{-5} g/ml crystalline lyophilized trypsin (Worthington Biochemical; 245 U/mg) and 5 × 10^{-6} g/ml deoxyribonuclease I (Worthington

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Biochemical; 9.1 × 10^4 U/mg) in a Ca^{2+}- and Mg^{2+}-free phosphate-buffered balanced salt solution containing (in mM) 116.0 NaCl, 5.4 KCl, 0.44 NaH_2PO_4, 0.95 NaHPO_4, and 5.6 dextrose. A pH of 7.3 was obtained by addition of 1 M HCl or 1 M NaOH.

The maintenance medium contained 20% medium 199 (GIBCO), 4% fetal bovine serum (GIBCO), and 2% horse serum (Kansas City Biological) in a bicarbonate-buffered balanced salt solution. Final concentrations were (approximately, in mM) 116.0 NaCl, 1.3 KCl, 1.8 CaCl_2, 0.8 MgSO_4, 0.9 NaH_2PO_4, 20.0 NaHCO_3, 0.8 MgSO_4, and 5.5 dextrose. Gentamicin sulfate (Schering, Garamycin; 10 mg/ml) was added to a final concentration of 5 × 10^{-5} g/ml.

The enzyme-inactivating medium was similar to the maintenance medium, except for 0% fetal bovine serum, 10% horse serum, and 4 mM KCl (approximately). All solutions were filtered with a 0.22-μm-diameter-pore-size sterile filter.

Electrophysiology

After 48–96 h in culture, the aggregates were transferred to a circular (35 × 10-mm) plastic tissue culture dish (Corning). A thin layer of mineral oil (Klearol, Witco) was poured on top of the medium to prevent evaporation. The bathing medium was gassed from above with 5% CO_2-10% O_2-85% N_2. Temperature was maintained at ~36 ± 1°C. The bicarbonate buffer maintained the medium at a pH of 7.2–7.3. Under such conditions, >96% of the aggregates show spontaneous rhythmic activity. Most of the aggregates studied had a diameter of ~175 μm and contained ~1,500–2,000 cells.

Electrical activity was recorded using borosilicate microelectrodes filled with 3 M KCl (typical microelectrode resistance 40–60 MΩ). Transmembrane potential was recorded, using an amplifier with negative capacitance compensation, to the nearest 0.25 mV. Action potential duration (APD) was measured at 90% repolarization. The bathing medium was kept at virtual ground by coupling to a current-to-voltage converter and by an automated computer system. Magnetic tapes were played back at 15 impulses/s, and the voltage waveform was sampled at 1 kHz by an IBM-compatible 386 computer through an analog-to-digital interface (Omega). Interbeat intervals were calculated from the digitized waveform by a pattern recognition program (Alembic Software). Computer programs were written to carry out further analysis of the interbeat intervals. Experimental traces were printed on a laser printer (HP Laserjet III) through a graphing package (Grapher).

Theoretical Model

Since the pioneering work of van der Pol and van der Mark (23), simple systems of ordinary differential equations have been used to model qualitative features of biologic oscillators (6, 9, 29). We have chosen a piecewise linear approximation to the van der Pol equations that contains a stable oscillating solution, a limit cycle, to represent the cardiac cycle. For technical details concerning the mathematics, see the APPENDIX.

The theoretical model is designed to capture the important qualitative properties of overdrive suppression in a schematic fashion. The main assumption of this work is that overdrive suppression arises as a consequence of a hyperpolarizing (outward) current that is induced by action potentials. Although we imagine that this current is associated with the transport of positive ions from the intracellular space to the extracellular space during the cycle, we develop the theoretical model in a general way that is consistent with a number of different ionic mechanisms. Therefore the present simplified theoretical model can provide a complementary approach to traditional ionic modeling and represents an important step in our understanding of overdrive suppression.

To carry out this task, the equations for the cardiac oscillator are modified to include a history-dependent hyperpolarizing current. The prolongation of the intrinsic cycle length after rapid stimulation is primarily due to a decrease in the slope of diastolic depolarization and, to a lesser extent, to changes in APD, maximum diastolic potential (MDP), and threshold potential (30).

The differential equations that we adopt for the periodically stimulated cardiac cells are as follows

\[
\frac{dV}{dt} = \frac{-1}{\epsilon} [y - f(V)]
\]

\[
\frac{dy}{dt} = a(V) - \beta \frac{Z}{Z + k}
\]

\[
\frac{dZ}{dt} = -\gamma \frac{Z}{Z + k} + \Delta Z \delta(t - t_{end})
\]

was repeated for several different amplitudes of stimulation to investigate the relationship between overdrive suppression and the intensity of the stimulus.

Overdrive suppression at different frequencies. Trains of 50 or 100 stimuli were delivered at different stimulation periods (T_s), with a rest of 30 s between successive trains to allow the cycle time to return to control. The period of stimulation was automatically decremented. Different stimulation strengths were also used to investigate the relationship between overdrive suppression and the intensity of the stimulus as well as the entrainment rhythms. The measured time intervals were normalized following the procedure described in the previous protocol.

Data Analysis

Off-line analysis was carried out on the digital oscilloscope and by an automated computer system. Magnetic tapes were played back at 15 impulses/s, and the voltage waveform was sampled at 1 kHz by an IBM-compatible 386 computer through an analog-to-digital interface (Omega). Interbeat intervals were calculated from the digitized waveform by a pattern recognition program (Alembic Software). Computer programs were written to carry out further analysis of the interbeat intervals. Experimental traces were printed on a laser printer (HP Laserjet III) through a graphing package (Grapher).
where V(t) corresponds to the experimentally observed transmembrane voltage, y controls the timing of the phases of the action potential, and Z is the variable associated with the history dependent hyperpolarizing current. The properties of the oscillation in the absence of Z are determined by the piecewise linear functions f(V) and a(V) (see APPENDIX). Finally, \( \varepsilon, \beta, \gamma, \) and \( \Delta Z \) are positive constants. \( \delta \) is the Dirac delta function, and \( t_{up} \) represents the time of upstroke of the action potential.

The physical interpretation of Eq. 1 is as follows. If we first fix \( Z = 0 \), there will be a stable oscillation of \( V \) and \( y \). For \( 0 < \varepsilon < 1 \), the oscillation is similar to a cardiac action potential with periodic rapid increases in \( V \) that we associate with the successive onsets of the action potential. Now consider what happens when \( Z \) is allowed to vary. The onset of the action potential leads to an instantaneous increment, \( \Delta Z \), of the factor \( Z \). Meanwhile, during the entire cycle, the level of \( Z \) is reduced following some \( Z \)-dependent rate. There is an associated term, \( -\beta [Z/(Z + k)] \), influencing the dynamics of \( y \) in the second equation. This term prolongs the duration of the depolarizing (pacemaker) phase of the cardiac cycle and, to a lesser extent, decreases the duration of the plateau of the action potential. Therefore the removal of \( Z \) can be associated with a hyperpolarizing current, where the magnitude of the current is proportional to \( Z/(Z + k) \) (see APPENDIX).

Numerical simulations were carried out by integrating Eq. 1 with use of a fourth-order Runge-Kutta method. To eliminate transients, initial conditions were chosen to lie on the limit cycle. The parameters of Eq. 1 were adjusted for each aggregate studied with use of the method described in the APPENDIX.

RESULTS

Overdrive Suppression at Fixed Stimulation Frequency for Different Numbers of Stimuli and Different Stimulus Intensities

In this protocol, the aggregates were stimulated for several different durations, maintaining a constant stimulation frequency. Figure 1A shows a typical experiment from an aggregate with \( T_0 = 520 \text{ ms} \) maintained at a stimulation period of 300 ms (\( \approx 0.58 T_0 \)). Initiation of the drive is generally followed by a transient depolarization, which may reflect changes in ionic gradients across the membrane. A hyperpolarization (increase in MDP) can sometimes be observed during the longer (1 min at 3 Hz) drives in spontaneously beating embryonic chick heart cell aggregates (20) but was not clearly present in our experiments. After 4, 15, and 50 stimuli, the first interbeat interval after the drive, \( T’ \), was prolonged by 20, 70, and 160%, respectively, over control.

The results of periodic stimulation for the same number of stimuli and the same stimulus periods in the theoretical model are shown in Fig. 1B. The degree of postdrive suppression of activity is roughly comparable to that in the experimental system. However, because the time-dependent process described in Eq. 1 does not influence the geometry of the limit cycle, no drive-induced hyperpolarization in MDP is found in the theoretical results (see APPENDIX).

Figure 2 illustrates how overdrive suppression is induced in the theoretical model during periodic stimulation (1, 4, and 15 stimuli) at a rate faster than control. The top traces show \( V(t) \); the corresponding changes in the level of \( Z \) are presented in the bottom traces. The control cycle length is 500 ms, and the stimulus period is 300 ms. The upstroke phase of the action potential is associated with a significant increase (\( \approx 30\% \)) in the level of \( Z \). Under control conditions (before stimulation), the same quantity of cations \( Z \) is removed by the electrogenic mechanism active during the entire cycle.

During periodic stimulation, increased action potential frequency results in accumulation of \( Z \), which stimulates the electrogenic mechanism, hence reducing the slope of diastolic depolarization. As in Fig. 1, the apparent decrease in APD during overdrive in the model is due to the geometry of the limit cycle (see APPENDIX). After cessation of stimulation, the level of \( Z \) decays because of

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1 The Dirac delta function \( \delta(s - s_0) \) is defined by its assigned properties: \( \delta(s - s_0) = 0 \) for any \( s \neq s_0 \) and \( \int_{-\infty}^{\infty} \delta(s - s_0) f(s) \, ds = f(s_0) \) for any function \( f \) continuous in the argument \( s \).
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Fig. 2. Numerical simulations of model showing relationship between overdrive suppression and level of $Z$. Parameters in model were adjusted to obtain control cycle length of 500 ms. Trains of 1, 4, and 15 stimuli ($A$, $B$, and $C$, respectively; $T_s = 300$ ms) were applied (1:1 entrainment), and resulting traces for $V(t)$ and $Z(t)$ (in units of $k$) are presented. Under control conditions, level of $Z$ varies by ~30% within cycle. Overdrive suppression is due to increased magnitude of $Z$-sensitive electrogenic current, because high action potential frequency during periodic stimulation causes accumulation of $Z$. This overdrive suppression decays subsequently, and within 10–15 s control activity is restored. Calibration bars as in Fig. 1.

Fig. 3. Composite of overdrive suppression as a function of stimulation time for atrial aggregates $AK61$ and $AK71$ stimulated with $T_s = 310$ ms and $T_s = 300$ ms, respectively. Interbeat intervals after drive, normalized to control cycle length ($T'/T_o$), are shown for increasing stimulus train durations. After drive, 1st cycle length is the longest, and normal activity is restored after 10–30 s. $A$: experiments; $B$: model simulations.

the increased extrusion via the hyperpolarizing current and the slowing of spontaneous activity (overdrive suppression). After 15 stimuli, the twofold increase in the level of $Z$ is associated with a 50% increase in the cycle length. Six seconds after cessation of stimulation, the level of $Z$ is only 10% above normal and control activity has almost resumed.

A composite of overdrive suppression, expressed as $T'/T_o$ vs. stimulation time, is shown in Fig. 3 for two different atrial aggregates stimulated with $T_s = 300$ and $T_s = 310$ ms. The postdrive pause developed slowly with the number of stimuli applied. As the number of stimuli applied increased further, the postdrive prolongation sometimes showed a tendency to saturate (30). Fig. 3B shows the results of simulation. There is good agreement between the numerical simulation and experimental data concerning the magnitude of the overdrive effect and its dependence on the number of stimuli applied. In the numerical simulation, however, the decay rate of overdrive suppression is initially too slow and then becomes too fast.

During sustained periodic stimulation at a fixed frequency, the observed entrainment pattern is often a function of the stimulus intensity (11, 12, 14, 30). For example, as the amplitude of the stimulus is varied while the period of stimulation is maintained constant, different types of $N:M$ locking between the stimulator and the preparation can be observed. The traces in Fig. 4 were obtained by maintaining a constant period of stimulation ($T_s = 145$ ms), but with stimulation intensities of ~18 nA in $A$, ~28 nA in $B$, and ~40 nA in $C$. As the stimulation intensity increases, there are different coupling patterns between the stimulus and the aggregate with 2:1 locking in $A$, 3:2 locking in $B$, and 1:1 locking in
OVERDRIVE SUPPRESSION OF HEART CELL AGGREGATES

Fig. 4. Recordings showing dependence of overdrive suppression on action potential frequency. A 180-µm-diam atrial aggregate with control cycle length of 470 ms (aggregate AK71) was stimulated with trains of 50 pulses of fixed period \(T_s = 145\) ms but different stimulus intensities. A: 2:1 locking, pulse amplitude \(\sim 18\) nA; B: 3:2 locking, stimulus intensity \(\sim 28\) nA; C: 1:1 locking, stimulus amplitude \(\sim 40\) nA. For a fixed rate of pacing, overdrive suppression is directly proportional to action potential frequency. For clarity, not all pulses are shown. Calibration bars as in Fig. 1.

C. The changes in the locking ratio are associated with changes in the overdrive suppression. After stimulation leading to 1:1 phase locking, the postdrive prolongation reached 340% over control, but this was reduced to 120% over control after 3:2 locking and to 70% over control after 2:1 locking. Similar results were obtained in six other preparations. This demonstrates that it is the frequency of the action potentials, rather than the period of stimulation, that is most critical in determining the magnitude of the overdrive suppression.

Overdrive Suppression at Different Frequencies

During periodic stimulation, different coupling rhythms between the stimulus and the preparation can be observed by changing stimulation intensity or stimulation frequency (11, 12, 14, 30). In the range of 1:1 entrainment during periodic stimulation with \(T_s < T_0\), there is overdrive suppression, where the increase in the magnitude of the slowing of the intrinsic rate is inversely proportional to \(T_s\). This is illustrated in Fig. 5, which shows three traces recorded from a 180-µm 7-day-old atrial aggregate with \(T_0 = 520\) ms. The aggregate was subjected to 50 stimuli for 145, 250, and 355 ms. In all three cases, there was stable 1:1 entrainment between the stimulator and the aggregate. After the drive, the first interbeat interval was prolonged by 300, 140, and 40%, respectively over control. Thus the postdrive prolongation after a fixed number of stimuli increases as the period of stimulation decreases, provided there is a maintained constant rhythm.

An interesting property of the experimental preparation is that it can be entrained in 1:1 fashion to periodic depolarizing stimuli with \(T_s > T_0\). However, this effect is much more difficult to observe than the 1:1 entrainment with \(T_s < T_0\) and could be measured in four preparations only (Fig. 6). In these cases, after cessation of stimulation, the intrinsic rate is slightly elevated, an effect that has been called underdrive acceleration (25). The importance of this effect is that it indicates a contribution of an electrogenic hyperpolarizing current, even during control conditions, which is consistent with the theoretical model.

As the stimulation frequency increases, maintaining the stimulation intensity fixed, there is typically a critical stimulation frequency that sets the fastest rate at which 1:1 entrainment can be maintained (11, 14, 30). In the 1:1 entrainment zone, the length of the first beat after the drive is inversely proportional to the period of pacing. At faster stimulation frequencies, there are \(N:M\) rhythms with \(N > M\), as in Fig. 4, where the magnitude of the overdrive effect decreases as a consequence of the dropped beats. In all the preparations studied, this "peaking" phenomenon was related to sudden changes in action potential frequency resulting from the transition from \(N:M\) to \(N':M'\) phase locking with \(N'/M' > N/M\). This is illustrated in Fig. 7, A and B, which shows the duration of the first beat after overdrive stimulation in a single aggregate at two stimulation intensities for
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pattern, proportional to the duration of the stimulation and inversely proportional to the period of stimulation. These findings are consistent with previous observations in chick heart cell aggregates (20, 30) as well as studies in a number of different preparations, including

50 stimuli. At the higher stimulation intensity (Fig. 7, A and B, right), the 1:1 entrainment was maintained for shorter stimulation periods ($T_s/T_0 = 0.3$), whereas with the weaker stimulation intensity (Fig. 7, A and B, left), the 1:1 entrainment was maintained until $T_s/T_0 = 0.45$. Figure 7C shows data in the same format superimposed for six different aggregates, including Fig. 7, A and B, with use of different stimulus amplitudes. For each aggregate, the postdrive prolongation and the period of stimulation have been normalized to the respective $T_0$. For stimulation periods where 1:1 entrainment was found for all six aggregates, overdrive suppression, scaled to the intrinsic cycle length, was approximately the same, independent of the preparation and the stimulus strength.

Figure 8 is a composite of the first postoverdrive cycle length after 50 stimuli as a function of the period of the stimulation for five different aggregates superimposed on the theoretical simulation. The measured time intervals are normalized to $T_0$. The parameters used in the numerical simulation were set for each aggregate according to the procedure described in the appendix. The values used in the numerical simulation are presented in Table 1. In all five aggregates, there is a similar dependence of overdrive suppression on the period of stimulation (scaled to intrinsic cycle length). This behavior is consistent with the numerical simulation of the theoretical model.

DISCUSSION

We have documented the effects of stimulation history on the overdrive suppression of spontaneous activity of chick atrial heart cell aggregates. The magnitude of overdrive suppression is, for a given entrainment
OVERDRIVE SUPPRESSION OF HEART CELL AGGREGATES

Table 1. Summary of parameters used in numerical simulations

<table>
<thead>
<tr>
<th>Aggregate</th>
<th>(T_0)</th>
<th>(APD)</th>
<th>(T'_{(\phi = 0.5)}/T_0)</th>
<th>(a_4)</th>
<th>(a_{APD})</th>
<th>(\Delta Z)</th>
<th>(\gamma)</th>
<th>(k)</th>
<th>(k/s)</th>
<th>Stimulus Amplitude, mV</th>
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<td>AK71</td>
<td>520, 80</td>
<td>1.07</td>
<td>5.68, 9.09</td>
<td>0.41</td>
<td>1.33</td>
<td>90 and 100</td>
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<tr>
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<td>6.17, 11.68</td>
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<td>1.44</td>
<td>93</td>
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\(T_0\), control cycle length; \(APD\), action potential duration; \(T'\), 1st interbeat interval after drive; \(a_4\) and \(a_{APD}\), positive constants related to duration of phase 4 and APD, respectively; \(\Delta Z\) and \(\gamma\), positive constants; \(k\), parameter that sets scale of \(Z\) (variable associated with history-dependent hyperpolarizing current).

Fig. 8. Composite of 1st cycle length after 50 stimuli as a function of period of stimulation for 5 different aggregates (filled symbols) superimposed on theoretical stimulation (solid line). Data in \(E\) and \(F\) were obtained from the same aggregate with use of 2 different stimulus amplitudes. See Table 1 for values of parameters used in simulations for each aggregate (AK78, AK34, AK36, AK70, AK71).

Table 1. Summary of parameters used in numerical simulations

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\(T_0\), control cycle length; \(APD\), action potential duration; \(T'\), 1st interbeat interval after drive; \(a_4\) and \(a_{APD}\), positive constants related to duration of phase 4 and APD, respectively; \(\Delta Z\) and \(\gamma\), positive constants; \(k\), parameter that sets scale of \(Z\) (variable associated with history-dependent hyperpolarizing current).

Purkinje fibers in sheep (24, 25) and dogs (8, 24, 25) and sinoatrial node in rabbits (19, 21), guinea pigs (10), and humans (13). In chick heart cell aggregates and other preparations, very fast pacing may lead to a partial block of activity (11, 30). Under such circumstances, for a fixed number of stimuli, overdrive suppression decreases with increasing degree of block (i.e., lower action potential frequency). This is in agreement with the idea that the frequency of action potentials is the major determinant of the postdrive prolongation in the cycle length (24, 25).

The development of the theoretical model was motivated by a desire to present a relatively clear mathematical picture based on physiologically plausible assumptions and in reasonable agreement with qualitative experimental evidence. Our formulation offers several advantages: 1) the model is based on a two-dimensional
approximation to the cardiac oscillator, which is not
preparation specific and may therefore be applicable to a
wide class of biologic systems; and 2) this comparatively
simple model can represent a useful step toward the
integration of an overdrive-inducing mechanism in a
high-dimensional ionic model, because it captures most
of the qualitative aspects of overdrive suppression in
this preparation.

Overdrive suppression has been related to several
mechanisms, such as the stimulation of the Na-K pump
via an increased level of internal sodium (3, 4, 7, 17, 20,
24, 25), accumulation of potassium outside the cells (15,
24), augmented uptake of calcium (10, 19), and release
of neuromediators (27). In embryonic chick heart cells,
overdrive suppression was found to be reduced by
ouabain, an extracellular blocker of the Na-K pump (17,
20). Because the Na-K pump plays a major role in
overdrive suppression in chick heart cell aggregates, it is
tempting to believe that, for this preparation, the theo-
retical model is a crude approximation to the mecha-
nisms underlying the influx (fast sodium current) and
the active transport (sodium pump) of sodium ions.

A connection can be made between the model and the
induction of overdrive suppression by assuming that the
influx of cations Z into the cell during the upstroke
phase of the action potential represents the influx of
sodium ions. To maintain a constant beat-to-beat level
of intracellular Z, this same quantity of ions Z is
extruded via some Z-sensitive (Michaelis-Menten kinet-
ics) electrogenic mechanism active during the entire
cycle. Increased entry of cations Z during fast pacing
(higher action potential frequency) activates the elec-
trogenic mechanism, which in turn slows diastolic depolar-
ization, transiently suppressing automatic activity. Af-
after the drive, the intracellular level of Z is gradually
restored and the cycle length returns to control. After a
fixed number of stimuli, the postdrive prolongation is
longer for faster stimulation rates. Thus the theoretical
model is consistent with a body of experimental results
that has accumulated over the past 25 years (13, 24, 25).

To be able to account for the overdrive suppression
following a single premature action potential, we must
assume a very significant variation ($\leq 30\%$) in the level
of Z during the cycle. The present theoretical model does
not incorporate any assumptions concerning the struc-
ture and the geometry of the intracellular space. Conse-
quently, one may suppose that the large changes in the
level of Z remain confined to a partition of the intracellu-
lar space that is available to the Z-sensitive electrogenic
mechanism. In fact, to properly describe the Na/Ca
exchange in excitable cells, several authors suggest a
hypothetical compartmentalization of the intracellular
space available to incoming sodium ions (2, 16). Al-
though the existence of this “fuzzy space” remains
unproven, it offers a possible explanation for the large
changes in the level of Z we must assume in the
theoretical model.

These considerations should be useful in developing a
detailed ionic model for this system. This type of model
is needed to overcome some of the limitations of the
current model. For example, this simplified model does
not adequately reproduce the experimentally observed
APD or phase-resetting behavior. There is shortening of
the APD during overdrive in the experiment and in the
model (Fig. 1). In the model, the APD is shortened when
a strong stimulus is applied early during phase 4,
causeing the trajectory to join the downward branch of
the limit cycle toward the end of the action potential (see
APPENDIX). In the real system, the APD depends on
complex interactions between inward and outward cur-
rents. Similarly, as discussed in detail elsewhere (14),
accurately reproducing experimentally observed phase
resetting is a difficult challenge but one that must be
confronted by accurate ionic models.

The current theoretical model allows one to predict
other types of kinetic behaviors that might arise from
changes in parameters. Previous studies have found
that the magnitude of the overdrive suppression satu-
rates after prolonged stimulation (10, 24). However, in
our theoretical model, whether or not the overdrive
suppression saturates depends on the parameters for
the kinetics of the inflow and extrusion of Z as well as
the frequency of stimulation. When very-high-frequency
stimulation is applied, the influx of cations Z may exceed
the extrusion capacity of the fully activated electrogenic
mechanism. Under these conditions, provided that 1:1
entrainment can be maintained, the theoretical model
predicts that postdrive prolongation would not saturate
and that there could be very long prolongations until
activity resumed. This could be of potential clinical
relevance in the setting of prolonged supraventricular
tachycardia.

The current study may have implications in the study
of rhythms observed in other systems. For example,
sustained periodic stimulation at fast frequencies may
induce “fatigue” in the atroventricular node (1) and
changes in the Purkinje fiber conduction properties (8),
sometimes leading to a complex evolution of rhythms
(22). In patients undergoing the sinus node recovery
test, an unusually long postdrive suppression of activity
is often associated with sick sinus syndrome (13). Dur-
ing this clinical test, as the frequency of the stimulation
is increased, the postdrive pause reaches a maximum
and then diminishes (13). The present study suggests
that this peaking phenomenon may be related to changes
in action potential frequency during periodic stimula-
tion. However, the actual mechanisms responsible for
overdrive suppression in the sinoatrial node may be very
different from those in the atrial aggregates, and there
may be rate-dependent contributions from changes in
neurohumoral factors (27) as well as changes in sino-
atrial conduction during overdrive.

We have demonstrated time-dependent effects of
stimulation on the cycle length of spontaneously beating
atrial chick embryo aggregates. This study reports the
first extensive experimental and theoretical investiga-
tion of frequency-related qualitative changes in the
intrinsic cycle length after overdrive in embryonic chick
atrial heart cell aggregates. Our theoretical approach is
based on a system of ordinary differential equations, and
the hypothesis that overdrive suppression in this prepa-
ration is due to the activation of a hyperpolarizing
current via an increased action potential frequency has been used with reasonable success to reproduce the qualitative aspects of overdrive suppression observed experimentally. The results here should be useful in the development of more rigorous ionic models of the mechanisms of overdrive suppression.

APPENDIX

Theoretical Model

We provide technical details on the properties of the differential equation used to model the cardiac oscillator. The two-dimensional system of ordinary differential equations that is used to model the action potential in the absence of the hyperpolarizing current is based on a modified version of the van der Pol equation (6, 23) as follows

\[
\begin{align*}
\frac{dV}{dt} &= -\frac{1}{\epsilon} [y - f(V)] \\
\frac{dy}{dt} &= \alpha(V)
\end{align*}
\]

where \(f(V)\) and \(\alpha(V)\) are piecewise linear functions of \(V\) and \(\epsilon\) is a positive constant. For \(0 < \epsilon \ll 1\), Eq. A1 is taken as a prototypical example of a limit cycle oscillation with fast relaxation to the limit cycle. The parameters and functions in Eq. A1 were selected so that \(V(t)\) corresponds roughly to the experimentally observed transmembrane voltage. For the heart cell aggregates, we assume that

\[
\begin{align*}
V &= \frac{20 + 4}{V < -60} \\
f(V) &= -\frac{V}{80} + \frac{1}{4}, -60 < V < 20 \\
V &= \frac{20}{V \geq 20}
\end{align*}
\]

and \(\alpha\) is a piecewise constant function of \(V\), such that

\[
\alpha(V) = \begin{cases} 
\alpha_4, & V < 0 \\
-\alpha_{APD}, & V \geq 0
\end{cases}
\]

where \(\alpha_4\) and \(\alpha_{APD}\) are positive constants related to the duration of phase 4 (diastolic depolarization) and APD, respectively (see below).

A representation of the limit cycle in the \(V - y\) plane (phase plane) is shown in Fig. 9A. The phase plane is divided into several regions corresponding to the phases of the cardiac cycle: the upstroke (region I), the plateau of the action potential (region II), the repolarization (region III), and the diastolic depolarization (region IV). The solid line represents the \(V\) nullcline, i.e., the set of points such that \(dV/dt = 0\). The dotted line shows the trajectory of a phase point as it travels on the limit cycle. Provided \(0 < \epsilon \ll 1\) (we will assume in what follows that \(\epsilon = 25,000^{-1}\)), the cycle can be divided into two phases: the action potential and phase 4. The durations of the action potential, \(t_{APD}\), and phase 4, \(t_4\), can be found by direct integration of Eq. A1. The duration of phase 4 is the length of time for \(y\) to increase from 0 to 1. Because \(dy/dt = 1/\alpha_4\) during phase 4, we immediately find

\[
t_4 = \frac{1}{\alpha_4}
\]

Similarly

\[
t_{APD} = \frac{1}{\alpha_{APD}}
\]

The relationship between \(\alpha_4\), \(\alpha_{APD}\), and \(V\) is summarized in Fig. 9B. An example of an action potential generated by this equation is shown in Fig. 9C. In numerical simulations, the model is driven by periodically adding a positive (depolarizing stimulus) constant to \(V\). Because the limit cycle is strongly attracting, high-amplitude stimulation at an early phase of the cycle may elicit a premature action potential with shortened duration (see Figs. 1 and 2). Although this is inconsistent with
experimentally measured behavior, changes in APD play a negligible role in overdrive suppression in this preparation (30).

In the presence of the hyperpolarizing current associated with $Z$, the dynamics are given by Eq. 1, which is repeated here, with the substitution $g(Z) = Z/(Z + k)$

$$\frac{dV}{dt} = \frac{1}{\epsilon} [y - f(V)]$$

$$\frac{dy}{dt} = \alpha(V) - \beta g(Z)$$

$$\frac{dZ}{dt} = -\gamma g(Z) + \Delta Z/s(t - t_{AP})$$

where $\beta$, $\gamma$, and $\Delta Z$ are positive constants and $t_{AP}$ is the time of the onset of the action potential. We assume that the removal of $Z$ follows Michaelis-Menten kinetics, so that $g(Z) = Z/(Z + k)$, where $k$ is a parameter that sets the scale of $Z$. As before, simulation of stimulation is carried out by adding a positive (depolarizing) constant $V$. We associate the start of the action potential with the time when the trajectory crosses the line $f(V) = -V/80 + \sqrt{4}$ while $V$ increases.

**Analysis of the Theoretical Model**

For each aggregate, it is necessary to specify six parameters: $\alpha_q$, $\alpha_{APD}$, $\beta$, $\gamma$, $\Delta Z$, and $k$. We briefly give our strategy for determining the values of these parameters and then give the details.

One of the experimental findings is that the slope of phase 4 after overdrive suppression may be quite small but is never negative. In the context of the theoretical model, this means that $\beta = \alpha_q$, so that with maximum overdrive the slope of phase 4 approaches 0. The parameter $k$ is used to set the scale of $Z$, so it is arbitrary. In the computations, we will express the concentrations of $Z$ in units of $k$. The parameters $\alpha_q$ and $\alpha_{APD}$ are related to the duration of phase 4 and the APD, respectively, with use of relations we give below, and are computed from measured values of these phases of the action potential appropriately modified by the effects of the overdrive term under control conditions. This leaves only two parameters, $\gamma$ and $\Delta Z$. We derive expressions that relate these two parameters to $T_0$, the cycle length that is found after a cycle induced by a stimulus delivered at phase $\phi$ during phase 4 of a control cycle $T'$ ($\phi$), and the mean value of $Z$ during a control cycle ($Z_0$).

For the computations that follow, in which it is necessary to compute the duration of various phases of the cycle, it is convenient to approximate the function $g(Z)$ by its mean value during a cycle. The justification for this approximation is based on the power series expansion around the mean value $Z$ during the cycle. We find that

$$g(Z) \approx \frac{Z}{Z + k} + \frac{k}{(Z + k)^2} (Z - \bar{Z}) + \cdots$$

Comparison of the magnitudes of the first terms shows that the first-order term is $\approx 10$ times smaller than the zeroth-order term as long as $|Z - \bar{Z}| < 0.4k$. Under control conditions or after a single premature action potential, the changes in the level of $Z$ are at most of the order of 0.5$k$ for all the aggregates studied (Table 1, Fig. 2). During the cycle, we can therefore approximate the function $g[Z(t)]$ by

$$g[Z(t)] \approx \frac{Z(t)}{Z + k}$$

The duration of phase 4 (diastolic depolarization), $t_4$, can be calculated from the above equations. The duration of phase 4 is determined by the integral

$$1 = \int_0^{t_{4}} dy - \int_0^{t_{4}} ds [\alpha_q - \alpha_{APD} g(Z(s))]$$

Because at the end of phase 4 we have $y(t) = 1$, the duration of phase 4 can be approximated

$$t_4 = \frac{1}{\alpha_q - \alpha_{APD} g(Z)}$$

A similar expression can also be obtained for the APD

$$APD = \frac{1}{\alpha_{APD} + \alpha_{APD} g(Z)}$$

The expressions for the effects of overdrive stimulation of the heart cell aggregates are in qualitative agreement with experimental observations: the duration of phase 4 increases while APD decreases. However, because $\alpha_{APD}$ is approximately twice the magnitude of $\alpha_q$ (Table 1), the effect of overdrive on APD (after the drive) is small compared with the effect on the duration of phase 4. Consequently, to facilitate computations, in the estimation of parameters we will assume that APD is constant, so that, under control conditions, the cycle length is

$$T_0 = APD + \frac{1}{\alpha_q - \alpha_{APD} g(Z)}$$

We now consider the effect of a single stimulus on the cycle length delivered at a phase $\phi$ during phase 4 of a control cycle that induces an action potential. The period of the cycle after the stimulus is $T''$, and the mean value of $Z$ during the cycle is $Z$. Because the resulting perturbation in the cycle length is small (experimentally measured: $\approx 7\%$ on average for $\phi = 0.5$),
we obtain (Fig. 2A)
\[
\bar{Z} = \bar{Z}_0 + \Delta Z(1 - \phi)
\]
\[(A7)\]

From Eq. A4, we find
\[
\frac{T' - APD}{T_0 - APD} = \frac{\alpha_4 - \alpha_4 g(\bar{Z}_0)}{\alpha_4 - \alpha_4 g(\bar{Z})} \cdot \frac{\bar{Z} + k}{\bar{Z}_0 + k}
\]
\[(A8)\]

Substituting for \(\bar{Z}\) from Eq. A7 into Eq. A8 and solving for \(\Delta Z\), we find
\[
\Delta Z = \frac{T' - APD}{T_0 - APD - 1} \left(\frac{\bar{Z}_0 + k}{1 - \phi}\right)
\]
\[(A9)\]

Under control conditions, the influx of cations, \(\Delta Z\), that enter during the action potential must balance the ions removed by the electrogenic pump. Consequently, from Eq. A2 we find
\[
\gamma g(\bar{Z}_0)T_0 = \Delta Z
\]
\[(A10)\]

Approximating \(g(\bar{Z}_0)\) from Eq. A3 and solving for \(\gamma\), we obtain
\[
\gamma = \frac{\Delta Z(\bar{Z}_0 + k)}{\bar{Z}_0 T_0}
\]
\[(A11)\]

To summarize the procedure used to set the parameters, we first use Eq. A4 to obtain \(\alpha_4\) from the experimentally measured duration of phase 4 (at control), \(t_s = T_0 - APD\). Equation A5 and the experimentally measured APD are then used to compute \(\alpha_{APD}\) as a function of \(\bar{Z}_0\). In the next step, by means of Eq. A9, we compute \(\Delta Z\) from the perturbed cycle length after a premature action potential elicited at phase \(\phi\). Finally, using the steady-state condition, we calculate \(\gamma\) (12). Therefore the degree of postdrive prolongation and the rate of the subsequent decay to \(T_0\) are controlled by a single parameter \((T')\) determined from single-pulse experiments, which suggests that the kinetics of dissipation of overdrive suppression are mainly governed by a steady-state condition for heat-to-heat variations in the level of \(Z\) (Eq. A10).

All the above parameters are a function of \(\bar{Z}_0\). Because we have no direct way of measuring \(\bar{Z}_0\) for each aggregate, we used the following method. A complete set of parameters was calculated for several values of \(\bar{Z}_0\). For each value of \(\bar{Z}_0\), the model was simulated to obtain a graph of overdrive suppression at different frequencies of stimulation. The resulting family of curves was superimposed on the corresponding experimental data (Fig. 10). For \(\bar{Z}_0 = 1.25k-1.75k\), there is good agreement between numerical simulation and experiment. Because qualitative aspects of overdrive suppression at different frequencies (in the 1:1 entrainment zone) are similar in all preparations (Fig. 7), an average value of \(\bar{Z}_0 = 1.50k\) was assumed for all the experiments considered.

Finally, the amplitude of the stimulus employed in the numerical simulation was adjusted by matching the range of the 1:1 entrainment zone in the numerical simulation with the corresponding experimental results. The values of the different parameters are summarized in Table 1. The simple geometry of the limit cycle and the mode of action of the time-dependent component that affects \(y\) but not \(V\) (Eq. 1) is responsible for the lack of effect of the hyperpolarizing current on MDP in the numerical simulations. Indeed, such a hyperpolarization of MDP is sometimes observed during prolonged (> 1 min at 3 Hz) overdrive in spontaneously beating embryonic chick heart cell aggregates (20) and may contribute to the postdrive pause. Because of the short (typically <30 s) duration of the drives used in our experimental protocols as well as the low value of membrane resistance at MDP, only a small effect of overdrive on MDP would be expected. This may explain the apparent lack of hyperpolarization of MDP observed in our overdrive protocols. In the context of the present theoretical model, such overdrive-induced changes in MDP could potentially be incorporated by letting the geometry of the limit cycle itself be influenced by increased action potential frequency. However, in view of the small magnitude of hyperpolarization observed in our experiments, such modifications are not warranted at the present time.

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