FLUCTUATIONS IN INTERBEAT INTERVAL IN RHYTHMIC HEART-CELL CLUSTERS

ROLE OF MEMBRANE VOLTAGE NOISE

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ABSTRACT Small clusters of ventricular cells prepared from 7-d chick heart maintain spontaneous, stationary, rhythmic beating in culture for many hours. For clusters containing 1–125 cells, mean interbeat interval ($\overline{IBI}$) is 0.45 ± 0.08 s and is independent of cell number (N), whereas the coefficient of variation of IBI (C) is proportional to $N^{-1/2}$. Because membrane voltage noise in such clusters would also be expected to vary as $N^{-1/2}$, we propose a model relating fluctuation in IBI (σ_{IBI}) to voltage noise (σ_V). A simplified model consisting of random voltage fluctuations superimposed upon a linear pacemaker depolarization of slope a is used to analyze the N-dependent shape of the IBI histogram. Values of σ_V derived from the relation $\sigma_{IBI} = \sigma_V/a$, or calculated from the skewness of the measured IBI histograms, both agree well with those extrapolated from steady-state noise recorded from resting heart-cell aggregates.

INTRODUCTION

The rhythmic beat of the heart is its most striking characteristic, but the rhythm is not as regular as it seems. Consecutive interbeat intervals recorded in the electrocardiograms from healthy individuals with normal sinus rhythms are not identical; rather, there are small, apparently random variations about an ideal mean. Gustafson et al. (1978) measured a standard deviation of 0.018 s in trains of heartbeats in a population of young adults with a mean interval of 0.728 s, i.e., a variation of ~2%. The random fluctuations in interbeat interval (IBI) of the heart may arise either from the multicellular nature of cardiac tissue, or from intrinsic properties of the excitable cardiac myocytes that comprise the heart. Our purpose here is to investigate the mechanism that underlies the variation in IBI in a model system of cultured heart tissue.

It is well known that individual myocytes isolated from the embryonic heart beat spontaneously in culture (Cavanaugh, 1955; Harary and Farley, 1963; Mark and Strasser, 1966; DeHaan, 1967; Goshima and Tonomura, 1969), and that when such cells come together into pairs or clusters, their rates of beating become synchronous (Cavanaugh, 1955; Goshima, 1969, 1970; DeHaan and Hirakow, 1972). Groups of thousands of heart cells formed by gyration culture into tissue-like spheroidal aggregates (Moscona, 1961; DeHaan and Sachs, 1972) establish electrically communicating junctions (McNutt, 1975; Ypey et al., 1979) and

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beat with a common coordinated rhythm with all of the cells experiencing the action potential for each contraction virtually simultaneously (DeHaan and Fozzard, 1975).

The variability in the synchronized beat rate of multicellular systems of coupled heart cells appears to depend upon the number of cells in the system. Jongsma et al. (1975) reported that single rate heart cells beat slowly and irregularly, whereas after 48 h in culture, when the cells had formed an interconnected synchronous network, the mean beat rate increased while the variability of consecutive intervals between beats decreased. Similarly, we have observed that chick heart-cell aggregates containing large numbers of cells beat regularly, while single cells and small clusters show greater variability in beat intervals.

In the present work we test systematically the hypothesis that the fluctuation in IBI is inversely related to the number (N) of coupled cells in a cluster under circumstances in which time in culture and other experimental variables are held constant. Furthermore, we propose a model to explain the observed decrease in fluctuation with larger values of N. The model is based upon the idea that membrane noise causes randomness in the time the preparation takes to depolarize from maximal diastolic potential (MDP) to threshold.

METHODS

The apical portions of heart ventricles were dissected from chick embryos incubated for 7 d at 37.5°C. The tissue was dissociated into its component cells in 0.05% trypsin (1:300, ICN Nutritional Biochemicals Div., Cleveland, Ohio) by the multiple cycle dissociation process described elsewhere (DeHaan, 1967; 1970).This procedure yields a suspension that is 85–90% single cells. An inoculum of 5×10^5 cells was added to 3 ml of culture medium (818A) contained in a 25-ml Erlenmeyer flask. The flask was gassed with 5% CO_2, 10% O_2, 85% N_2, sealed with a silicone rubber stopper, and placed on a gyratory shaker (62 rpm, 1¼-in. stroke) for 4 h at 37.5°C to allow aggregation to begin (Sachs and DeHaan, 1973). The contents of each flask were transferred to a plastic tissue culture dish (Falcon Plastics Co., Oxnard, Calif.; type 3001) on the warm stage of an inverted optics phase-contrast microscope (Nikon Inc., Instrument Div., Garden City, N.Y.). Temperature was controlled at 37.5 or 30.5°C (continuously monitored) and a mixture of 5% CO_2, 10% O_2, 85% N_2 passed through a toroidal gassing ring surrounding the dish, maintained at a constant atmosphere and a pH of 7.3. Nontoxic mineral oil (Klearol, Sonneborn Division, Witco Chemical Corp., N. Y.) layered over the medium in the dish prevented evaporation and provided an unclouded optical image of the cells. Medium 818A (DeHaan, 1970) contains (by volume) 25% M199 (Grand Island Biological Co., Grand Island, N. Y.), 2% heat-inactivated selected horse serum (K. C. Biologicals, Lenexa, Kans.), 4% fetal calf serum (Grand Island Biological Co.), and 0.5% gentamicin (Schering Corp., Bloomfield, N.J.) in K-free Earles balanced salt solution, made up as follows (in mM): NaCl, 116.0; $MgSO_4$, 0.8; NaH_2PO_4, 0.9; $CaCl_2$, 1.8; $NaHCO_3$, 26.2; and glucose, 5.5. The potassium concentration of the final medium was brought to 1.3 mM. Under these conditions, the cells and small clusters adhered firmly to the bottom of the culture dish in an hour or less and maintained continuous stationary beating for many hours or until experiments were terminated. With no external perturbations trains of hundreds or even thousands of intervals could be recorded. For recording from single cells, the freshly dissociated suspension was plated at low density (2×10^5 cells per plate) in 1.3 mM K medium for 24 h without previous gyration.

Beating activity of cells and clusters was monitored with the aid of a closed circuit video system (Fig. 1) modified from that described previously (Nathan et al., 1976). A closed circuit television camera (Panasonic model WV-400P, Matsushita, Yokohama, Japan), positioned at the photo-ocular of the microscope, projected the image onto a TV screen (Panasonic model TR413V). With a 20× objective (microscopic magnification 200×), the image magnification on the video screen was adjusted (1,500–3,000×) to allow only a small number of cell groups to come within the field of view. Beats were recorded by placing a phototransistor sensor (Fairchild semiconductor, Fairchild Industrial Products Div., Winston-Salem, N.C.; FP100) on the video screen over the edge of the image of a cell or cluster,

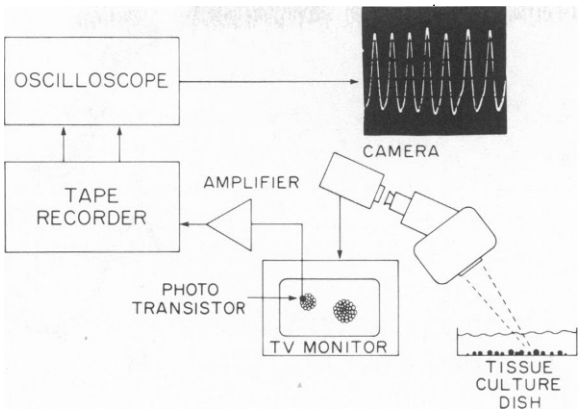

FIGURE 1 Block diagram of the recording apparatus. Insert: typical record from a cluster of 10–20 cells.

which changed its optical transmittance during each contraction cycle. Signals from the phototransducer were displayed on an oscilloscope (Tektronix, Inc., Beaverton, Oreg.; model R5103N/D13) and stored on magnetic tape (Hewlett-Packard Palo Alto, Calif., model 3960) at a tape speed of 15/16 ips (d.c., 325 Hz) for off-line analysis. The recorded signals were processed with a PDP 8/L computer (Digital Equipment Corp., Maynard, Mass.) with the aid of a Schmitt trigger that was used to determine the interval between beats. Before analysis some records were amplified and filtered with a Krohn-Hite 332 low-pass filter (Krohn-Hite Corp., Avon, Mass.) (cut-off frequency 20–30 Hz). The values for beat interval and beat variability from filtered and nonfiltered records never differed more than 10%. A standard computer program (DEC-LAB-8 software package; Digital Equipment Corp., Marlboro, Mass.) was used to computer interval histograms with a 5-ms bin width.

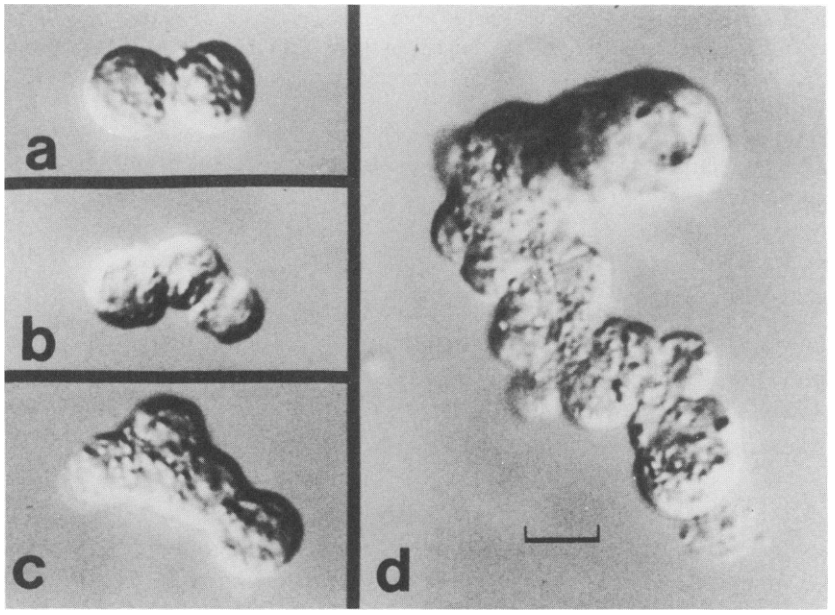

FIGURE 2 Representative cell clusters photographed with Nomarski optics after 4 h of gyration culture and ~90 min adhesion to the dish. *a–d* illustrate clusters containing 2, 3, 5, and 27 cells, respectively. Scale, 10 μm.

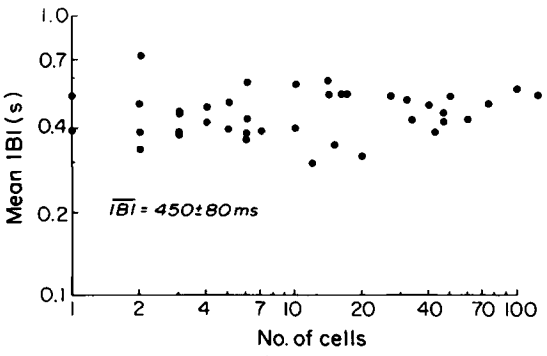

FIGURE 3

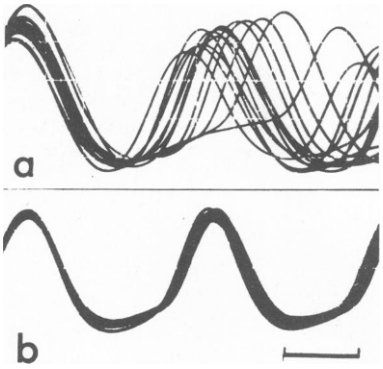

FIGURE 4

FIGURE 3 Mean interbeat interval of heart cell clusters in the range N = 1–125. A least-squares line fit through the data has a slope that is not significantly different from 0 (P = 0.60).

FIGURE 4 Phototransistor output recording of 15 consecutive interbeat intervals from two different cell clusters in the same culture dish under identical conditions. (*a*) 2-cell groups; $\overline{IBI}$, 0.465 s; C, 23.5. (*b*) 75-cell group; $\overline{IBI}$, 0.49 s; C, 3.9. Scale = 200 ms; K_o = 1.3 mM.

The number of cells (N) per cluster was determined directly from the microscope because it afforded a higher degree of resolution than the image on the video screen. Cells could also be viewed with bright-field or Nomarski optics. The mechanical excursion with each contraction of single spherical cells was very small after 1–2 h of adhesion to the dish, but increased with time in culture. For this reason optical recordings from single cells were made from cultures 24 h after plating. The number of cells in clusters of up to about 30 cells could be determined by inspection. In larger groups N was estimated by counting the nuclei visible under phase optics and by measuring the size of the parts of each cluster. In some of the larger preparations these estimates were later confirmed by nuclear counts of the same cluster after fixation and staining with hematoxylin. In these cases the two counts agreed within ±10%. In experiments that lasted several hours the clusters tended to flatten slightly after they adhered to the bottom of the culture dish, making it easier to estimate N.

Action potentials were recorded from aggregates of 50 to several thousand cells by using standard intracellular microelectrode techniques described previously (Nathan et al., 1976; DeHaan and Fozzard, 1975; DeHaan and DeFelice, 1978*b*).

RESULTS

After 4 h of gyration, the cell suspension contained small groups of 2 to more than 100 cells, as well as many singlets (Fig. 2). In every case all cells in a cluster beat synchronously. For clusters up to N = 125 mean spontaneous pulsation rate was not dependent upon N. The mean interbeat interval ($\overline{IBI}$) of 40 cell groups was 0.45 ± 0.08 s ($\bar{X}$ ± S.D.), as determined from 300–500 intervals from each cluster. The data do not vary systematically with cluster size (Fig. 3).

In contrast to $\overline{IBI}$, variance in IBI was markedly dependent upon N. Representative records of 15 consecutive intervals (Fig. 4) demonstrated that small clusters beat irregularly (Fig. 4 *a*) while larger groups of cells exhibited a regular and consistent rhythm (Fig. 4 *b*). Fluctuation in IBI may be described by the coefficient of variation (C):

$$C = \frac{\sigma_{IBI} \cdot 100}{\overline{IBI} - APD}, \quad (1)$$

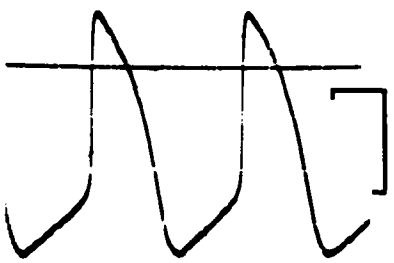

FIGURE 5

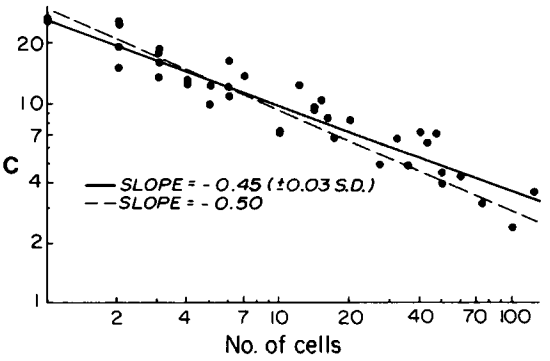

FIGURE 6

FIGURE 5 Action potentials recorded from a well-rounded cluster of ~100 cells. Vertical scale: 40 mV; horizontal scale 0.1 s.

FIGURE 6 Fluctuation in IBI, expressed as C, as a function of cell number (N).

where σ_{IBI} is the standard deviation of a series of intervals and APD is the action potential duration. Contraction cycle intervals such as those illustrated in Fig. 4 consist of a contraction phase that is approximately coincident with the membrane action potential, and a rest period during which the membrane voltage undergoes diastolic depolarization from the maximal diastolic potential (MDP) to threshold (Fig. 5). Measurement at 37.5°C of APD from 8 clusters and aggregates beating at 120–150 beats/min ($\overline{IBI}$ = 0.4–0.5 s) gave a mean value of 177 ± 27 ms and was independent of aggregate size over the range 50–1,400 cells. Therefore, the value of APD was rounded off and taken as 180 ms. As discussed below, this has the effect of ascribing the entire fluctuation in IBI to the diastolic depolarization phase of the contraction cycle.

The dependence of C on N is illustrated in Fig. 6, which includes values for the same 40 clusters represented in Fig. 3. The slope of the least-squares best fit line through the data,

TABLE I
MEAN IBI AND BEAT FLUCTUATION AS A FUNCTION OF TEMPERATURE

	37.5°C		30.5°C		37.5°C	
N	IBI	C	IBI	C	IBI	C
2	—	—	0.965	16.7	0.725	18.4
4	—	—	0.819	9.4	0.421	12.5
14	0.523	9.3	1.036	9.7	0.586	9.6
40	—	—	0.983	9.1	0.480	7.3
60	—	—	0.887	5.1	0.430	5.1
125	0.524	3.7	0.965	3.7	0.518	3.8
Mean	0.524	—	0.941 ± .08	—	0.527 ± .11	—

Trains of 300–500 beats were recorded from identified clusters at 30.5°C. The temperature was readjusted to 37.5°C, a period of 30 min was allowed for re-equilibration, and a like number of beats was then recorded from the same clusters. In two of the clusters (N = 14, 125), a prior recording had been made at the higher temperature about 2 h before the temperature drop to allow a comparison between the conditions before and after the low temperature phase.

plotted on a double log scale, is -0.45 ± 0.03. The broken line has a slope of -0.5 (see below).

Trains of intervals from 6 clusters were recorded at both 37.5° and 30.5°C. At the lower temperature, IBI was roughly double that at 37.5°C (Table I), but C remained virtually unchanged. Measured APD increased from 180 to 310 ms in action potentials recorded from a typical aggregate containing a few hundred cells, after shifting from 37.5 to 30.5°C. The two cell groups from which interval trains were recorded at 37.5°C both before and after the low-temperature period indicate that the effect of the temperature change on C and IBI was reversible, and illustrate the degree of stationarity of these preparations over a period of 6–7 h.

Interval histograms from 4 representative clusters are shown in Fig. 7. For single cells and very small groups the histograms are skewed toward longer intervals, whereas they appear Gaussian for $N > 30$. The smooth curves in Fig. 7 are theoretical probability density functions derived from the model described below.

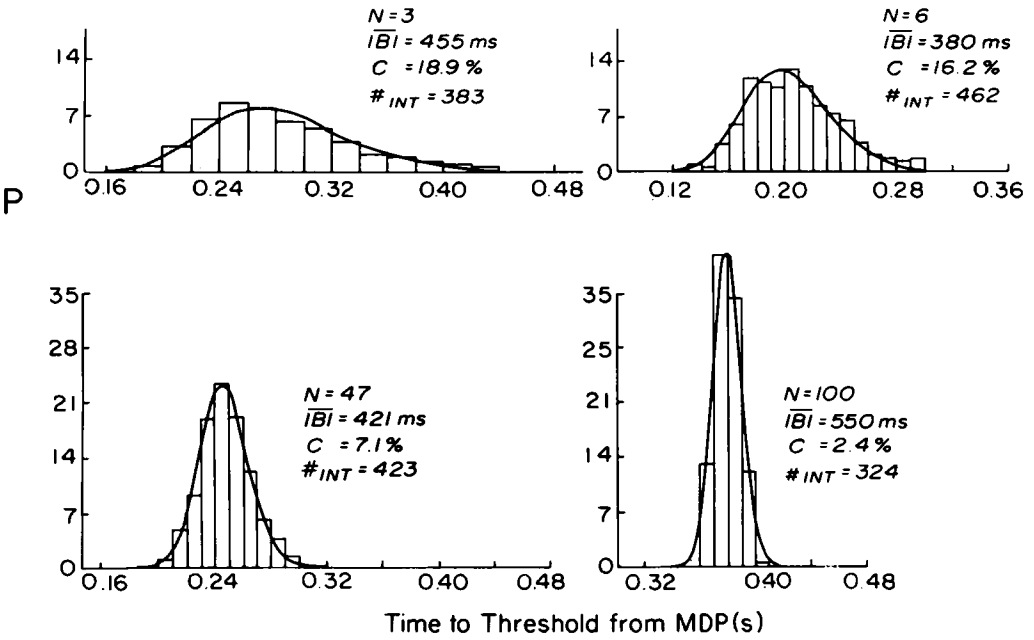

FIGURE 7 Interval histograms from 4 representative clusters. The smooth curves are best-fit probability density functions derived as described in the text. Threshold (V_T) is taken as 25 mV with respect to V_{MDP}. Slope of pacemaker depolarization (a) is calculated as $a = (V_T - V_{MDP})/(\overline{IBI} - APD)$. The remaining parameter of the model (D) was obtained by the best fit procedure using the χ^2 as the fit criterion. The values of D and $P\chi^2$ for each cluster were:

N	$D(V^2/s)$	$P\chi^2$
3	2.2×10^{-4}	0.45
6	1.8×10^{-4}	0.35
47	3.6×10^{-5}	0.75
100	3.4×10^{-6}	0.55

DISCUSSION

Sachs and DeHaan (1973) reported that heart-cell aggregates containing a few hundred to several thousand cells beat at a rate that was inversely proportional to aggregate size. The uniformity of $\overline{IBI}$ for the clusters employed in the present study indicates that the rate-setting mechanism is not influenced by cell number when $N \leq 125$. A comparison of the rate data of Sachs and DeHaan (1973) with that illustrated here (Fig. 3) suggests that tissue size begins to affect mean pulsation rate only when $N \geq 200$–400. The decrease in beat rate with size for these larger aggregates does not result from diminished electrical coupling among the cells. Action potentials or subthreshold voltage perturbations are experienced virtually simultaneously and without appreciable diminution by widely separated cells in such aggregates (Sachs and DeHaan, 1973; DeHaan and Fozzard, 1975). Even pairs of aggregates become tightly coupled and synchronize their beats across newly formed low-resistance junctions when they are pressed together for a time (Ypey et al., 1979). Sachs and DeHaan (1973) hypothesized that beat rate may be a function of total coupled cell surface, or, alternatively, that the rate-size relationship results from increasing accumulation of ions or metabolites in the intercellular clefts of progressively larger aggregates during activity. In clusters of fewer than 125 cells the surface of all cells should be accessible to the bulk extracellular medium with no appreciable diffusional restriction. When the diffusional path from the center of an aggregate to the bulk medium becomes too great, however, a condition much like that in intact ventricular muscle or cardiac Purkinje fiber may obtain, in which metabolites and ions accumulate in the intercellular spaces with each beat (Kline and Morad, 1976; Baumgarten and Isenberg, 1977). Both K^+ and H^+ are known to slow the spontaneous pulsation rate of isolated cardiac myocytes (Oliviera-Castro and Paes de Carvalho, 1970; Goshima, 1975). If K^+ or acid metabolites accumulate in the cleft spaces in the center of a large aggregate to a concentration greater than that in the bulk medium, the aggregate would beat more slowly than a smaller cell cluster in the same medium. Therefore, for clusters where $N = 1$–125, rate is apparently set by intrinsic membrane mechanisms such as voltage- and time-dependent conductances, rather than by a mechanism dependent upon tissue geometry. This contention is supported by the temperature Q_{10} of 3–4 for $\overline{IBI}$ (Table I) of small cell groups. A similar temperature dependence has been reported for other cultured heart-cell preparations (McCall, 1976) and has been ascribed to a direct effect on the pacemaker currents (Noble and Tsien, 1968).

Whereas the $\overline{IBI}$ of cell clusters was not dependent on N, beat-to-beat fluctuation in IBI clearly was (Fig. 4). This imperfect regularity of beating could result from spontaneous fluctuations in the contraction or relaxation times of the myofibrillar proteins during each beat, or from some aspect of the pacemaker mechanism such as stochastic variations in one or more of the pacemaker currents. The simple mechanism we propose here is that the increase in irregularity of beating in small clusters is associated with membrane voltage noise produced by random opening and closing of the pacemaker current conductance channels (Clay et al., 1979*a*). Various schemes have been introduced relating membrane noise to excitability (Lecar and Nossal, 1971; Clay, 1976; DeHaan and DeFelice, 1978*b*). A plausible model for a dependence of beat interval on voltage fluctuations is one in which membrane potential rises to threshold according to a random walk superimposed on a linear drift (Gerstein and Mandelbrot, 1964; Johannesma, 1968; Pernier and Gerin, 1975). In a similar vein, we suggest

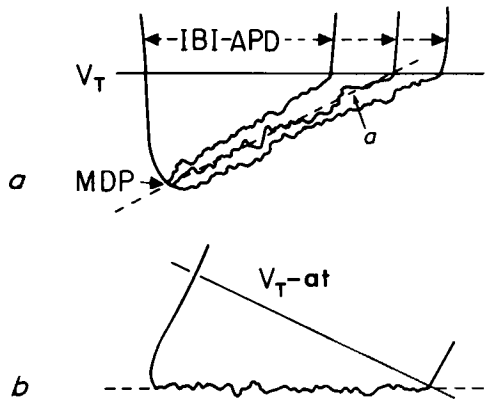

FIGURE 8 Schematic representation of the noisy drift of the membrane toward threshold (V_T) during pacemaker depolarization from maximum diastolic potential (MDP). (*a*) Three sample paths in which the membrane reaches V_T at different times given by the IBI-APD. The broken line (*a*) represents the mean depolarization path. (*b*) A simple transformation of the process in (*a*) in which the upward drift of the mean voltage path is replaced by a downward linear drift of threshold toward the constant membrane potential. The purpose of this transformation is to facilitate the analysis described in the text.

that random fluctuations in membrane voltage superimposed on the slope of the diastolic depolarization cause variations in the time it takes for the voltage to reach threshold (Fig. 8 *a*). If this hypothesis is correct C should be directly related to the rms voltage noise (σ_V) during the diastolic depolarization. Since the amplitude of the noise that is recorded from heart-cell aggregates is proportional to $R_i^{1/2}$ and R_i is proportional in turn to N^{-1} (Clay et al., 1979*a*), C should vary as $N^{-1/2}$. This is consistent with the simplest interpretation of the data summarized in Fig. 6. A second argument in favor of a dependence of C on membrane voltage noise derives from the temperature shift experiments (Table I). Conti et al. (1975) have shown that the rms amplitude of membrane current noise in nerve is nearly independent of temperature. Consequently, voltage noise would also lack a temperature dependence, at least for low frequencies limited by the membrane time constant. If heart membrane noise behaves similarly, C should be independent of temperature (see Table I).

We emphasize that the dependence of C on $N^{-1/2}$ assumes that all cells in a cluster are well coupled. That is, a single spontaneously beating cell of variable radius r would duplicate the result. For this hypothetical system the relation: $C \sim N^{-1/2}$ would transform to $C \sim r^{-1}$.

Two independent estimates of the amplitude of the voltage noise that underlies beat variability may be made from the IBI histogram of Fig. 7. One can be derived from the measurements of variance of IBI (σ_{IBI}) and the linear slope of the diastolic depolarization (a), by using the simple relation $\sigma_{IBI} = \sigma_V/a$. Between maximal diastolic potential (MDP) and threshold (V_T), membrane potential depolarizes about 25 mV (Fig. 5). The duration of the diastolic depolarization for the 100-cell cluster in Fig. 7 was 550 − 180 = 370 ms. Thus, a = 25/370 = 0.068 mV/ms. Since C = 2.4% for this preparation, $\sigma_V = a \cdot \sigma_{IBI}$ = 0.60 mV. A similar calculation for the 3-cell cluster gives σ_V = 4.7 mV (Table II).

The alternative measure of σ_V is available from an analysis of the skewness of the IBI histograms. As mentioned above, the noisy drift of the heart cell membrane toward threshold is similar in some respects to the situation in the synaptically excited nerve membrane.

TABLE II
CORRESPONDENCE OF σ_V PREDICTED FROM THE MODEL AND EXTRAPOLATED FROM VOLTAGE NOISE MEASUREMENTS ON LARGER AGGREGATES

σ_V	N	
	100	3
	mV	*mV*
$a \cdot \sigma_{IBI}$	0.6	4.7
diffusion model	0.7	5.8
extrapolated from membrane noise	1.1	6.4

Assuming that the duration of the diastolic depolarization is comparable to or less than the membrane time constant, the voltage drift can be treated as a random walk by simple diffusion theory (Gerstein and Mandelbrot, 1964; Johannesma, 1968). Because membrane conductance in the pacemaker voltage range is voltage dependent, τ_m increases as V_m depolarizes from MDP toward V_T (DeHaan and DeFelice, 1978*b*). The slope resistance of 7-d ventricular aggregates in 1.3 M K_0^+ is approximately 40 k$\Omega \cdot$ cm^2 from -90 to -75 mV and increases to 150 k$\Omega \cdot$ cm^2 for -75 to -60 mV (Shrier and Clay, 1979). Since the specific membrane capacitance is approximately 1 μF/cm^2 (Clay et al. 1979*a*), the RC time constant is comparable to the duration of diastolic depolarization from -75 to -60 mV. A small time-dependent current is also present in the pacemaker region (Clay et al., 1979*b*) which has properties similar to those of the i_{K_2} current in cardiac Purkinje fibers in low K_0^+.[1] The fully open IV relation of this current has a negative slope conductance throughout the pacemaker range of potentials which effectively increases the slope resistance and hence the RC time constant during pacemaker activity (Noble, 1975). That is, the entire duration of the diastolic depolarization may be comparable to the effective membrane time constant in a beating preparation. Consequently, we apply the simple diffusion model to the entire diastolic depolarization, and, as a first approximation, we neglect the voltage dependence of the slope resistance.

The mathematical problem we wish to solve is a random walk from maximum diastolic potential V_{MDP} to threshold potential V_T with a linear drift of slope a. This problem is equivalent to a random walk without the drift, where the threshold is $V_T - at$ (Fig. 8 *b*), t is time, and $t = 0$ corresponds to the initiation of the random walk at MDP. We set $V_{MDP} = 0$ and let $P(V, t)\mathrm{d}V$ be the probability for the membrane potential to be within the interval (V, $V + \mathrm{d}V$) at time $t > 0$, given that it was at zero membrane potential initially. We assume that P is a solution to the diffusion equation

$$\partial P/\partial t = D\partial^2 P/\partial V^2, \tag{2}$$

where $D = \sigma_V^2/\mathrm{RC}$ and RC is the mean membrane time constant (Wang and Uhlenbeck, 1945). When the membrane potential crosses threshold it always fires. That is, the threshold

[1]Shrier, A., and J. R. Clay. Work submitted for publication.

or boundary "absorbs" the potential so that $P(V = V_T - at) = 0$. The solution to Eq. 2 with this boundary condition can be obtained from the method of images (Daniels, 1969). The result is:

$$P(V, t) = \{\exp(-V^2/4Dt) - \exp[V_T a/D - (V - 2V_T)^2/4Dt]\}/(4\pi Dt)^{1/2}. \quad (3)$$

The probability that the membrane reaches threshold in any time interval $(t, t + dt)$ is given by the first passage time density function $P_F(t)$, which is mathematically equivalent to the IBI distribution. This function is given by

$$P_F(t) = -\partial/\partial t \int_{-\infty}^{V_T - at} P(V, t)\, dV. \quad (4)$$

Combining Eqs. 3 and 4 gives

$$P_F(t) = a \exp[-(V_T - at)^2/(4Dt)]/(4\pi Dt)^{1/2}. \quad (5)$$

In Eq. 5, t can be replaced by IBI without altering the meaning of the expression. This emphasizes that $P_F(t)\, dt$ is the probability that an interval of duration equal to IBI will occur in $(t, t + dt)$, where $V = V_{MDP}$ at $t = 0$.

Eq. 5 is a function of a, D, and V_T, and is indirectly related to $\overline{IBI}$ through the dependence of a on $\overline{IBI}$. V_T is 25 mV (Fig. 5), $\overline{IBI}$ is measured (Fig. 6), and a is determined from $V_T/(\overline{IBI}\text{-APD})$. Thus, the only undetermined parameter in Eq. 5 is D. This can be derived from a best fit of the equation to the IBI histograms. For large values of D, Eq. 5 is skewed toward larger intervals, while for smaller values it becomes very nearly Gaussian. Consequently, D can be chosen to cause the skewness of the curve generated by Eq. 5 to match that of the data in Fig. 7. The smooth lines through each histogram in Fig. 7 were computed from Eq. 5 by this procedure and thus determine σ_V^2. For the 100-cell cluster $D = 3.4 \times 10^{-6} V^2/s$; for the 3-cell group $D = 2.2 \times 10^{-4} V^2/s$. Taking the RC time constant to be 0.15 s (Shrier and Clay, 1979), we calculate that σ_V for $N = 100$ and for $N = 3$ correspond nicely to the estimates made with fewer assumptions directly from $a \cdot \sigma_{IBI}$ (Table II).

Although it was possible to record action potentials from cell clusters (Fig. 5), we were unable to measure steady-state voltage noise from these preparations to compare with the predicted values. The clusters always beat spontaneously upon impalement, even in the presence of tetrodotoxin (TTX). Since the input resistance (R_i) of a 100-cell cluster would be 50–250 MΩ in the pacemaker voltage range, even a small leak around the electrode would lead to an appreciable depolarizing current. This was not a problem for larger aggregates (R_i = 1–3 MΩ), which could be made quiescent with TTX after impalement with one or even two electrodes (DeHaan and DeFelice, 1978*a*).

As noted previously, the beat rate of larger aggregates ($N > 200$–400) is inversely proportional to r, although their cells are demonstrably well coupled (Sachs and DeHaan, 1973; DeHaan and Fozzard, 1975). When spontaneous beating in these preparations was suppressed with TTX, the degree of cell coupling could be measured as a function of signal frequency. The low-frequency noise ($f < 160$ Hz) from two widely separated cells in a quiescent aggregate exhibited a coherence function that approached unity (DeFelice and DeHaan, 1977). Moreover, R_i at constant potential was inversely proportional to r^3 (r = 50–150 μm), and the input capacitance was a direct function of r^3 (Clay et al., 1979*a*). Since the total membrane surface area was also proportional to r^3, we conclude that even large

aggregates may be modeled, at low frequencies ($f < 10$ Hz), with a lumped equivalent circuit in which series resistance is neglected (Clay et al., 1979*a*). These results indicate that low-frequency voltage noise recorded from aggregates arises from their intrinsic membrane properties, and that information obtained from such noise is applicable to small cell clusters.

A spheroidal heart cell aggregate containing ~3,000 cells (diam = 171 μm) at $V_m = -61$ mV exhibited a membrane resistance of ~55 k$\Omega \cdot$ cm^2 (Clay et al., 1979*a*). The rms voltage noise amplitude recorded from this preparation was 0.2 mV. Applying the $N^{-1/2}$ relationship, σ_V for a cluster of 100 cells should be 1.1 mV and for a 3-cell group, 6.4 mV (Table II). These extrapolations correspond well to the predictions of σ_V and σ_{IBI} and from the skewness of the IBI histograms.

Although our model of voltage noise superimposed on the depolarizing pacemaker potential has predictive value, we recognize that it fails to correspond to reality in several ways. (*a*) The diastolic depolarization is not a simple linear process. Impedance and voltage clamp analysis of 7-d heart-cell aggregates exposed to 3 μM TTX and 4.5 mM K_0^+ indicate that the current-voltage (IV) relation shows strong inward rectification and a region of negative slope conductance in the pacemaker voltage range (Clay et al., 1979*b*). That is, the slope resistance is a function of membrane potential in the pacemaker voltage range, as discussed above. (*b*) Stationary fluctuation analysis is not appropriate for a nonstationary system unless the frequency of the fluctuations is much greater than the mean rate of change. The stationary noise recorded from aggregates has its main power near 1 Hz (DeHaan and DeFelice, 1978). Thus, only a fraction of σ_V should affect σ_{IBI}, as seen in Table II. This argument is discussed in detail by Minorsky (1962) and Firth (1966). (*c*) Finally, the same processes that result in fluctuations in voltage around a mean V_m would presumably yield random variations about the mean V_T as well. However, we have not incorporated this complication in the present model.

Variability of contraction of cardiac tissue in vitro has also been studied by Jongsma et al., (1975) in embryonic rat heart cells plated at high density (5×10^5 cells/ml). They found that the beat rate increased as cells came into contact and that the irregularity relative to mean rate decreased. Although these authors were unable to assess the number of interconnected cells in their monolayer culture, the latter finding is consistent with our results. The effect on beat rate of tissue size they found cannot be readily compared with the results in the present study because of the different geometries of tissue, length of time in culture, preparative procedures, and, of course, the different species involved. Nonetheless, the finding of a decrease of C with tissue size from two laboratories using different tissues and preparative procedures suggests that the phenomenon may be a general one for electrically coupled cardiac cells.

Variability in IBI is clearly influenced by membrane noise, and the model described here approximates that dependence for the heart-cell membrane. Stochastic analyses of the Van der Pol oscillator (Clay, 1976) and the Hodgkin-Huxley nerve membrane model (Lecar and Nossal, 1971; Clay, 1977) can serve as the basis for a more detailed modeling approach to IBI variability when voltage clamp investigations of the heart-cell membrane currents have been completed. Ultimately, a rigorous description of the relationship between membrane noise and fluctuation in IBI will require direct measurements of single channel currents during diastolic depolarization.

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