Alternans in periodically stimulated isolated ventricular myocytes: Experiment and model

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> The alternating contraction of the ventricle is not a simple pathological phenomenon but rather a general physiological function, that makes its appearance in many circumstances, ... We have, in fact, to deal with a capacity of the cardiac muscle which enables the ventricle to go on with rhythmical contractions even under abnormal conditions.
>
> Muskens (1907–08)

INTRODUCTION

Beat to beat alternation in the intensity of the peripheral arterial pulse was first described using graphical recording techniques more than a century ago. This alternation is usually attributed to an alternation in the force of contraction of the left ventricle of the heart. Perhaps the simplest way in which alternation could occur is if there would be a simultaneous concordant alternation of the force of contraction in all cells of the left ventricle. In 1882 Gaskell put forth an alternative hypothesis stating that a beat to beat alternation in the intensity of the heart beat could be due to spatial inhomogeneities in contractile activity in different areas of the ventricle: 'The explanation, therefore, of this alternation in the force of the contractions must be sought for in the muscular tissue itself, and it seems to me that the

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most probable explanation is that a larger amount of tissue contracts when the beats are large than when they are small, and that therefore, in all probability, certain portions of the ventricle respond only to every second impulse, while other portions respond to every impulse'. A third possibility, that there could be a 2:1 response in two different subpopulations, with the conducted beat occurring on alternate beats in the two subpopulations, was suggested by Mines (1913).

With the advent of electrical recording techniques, electrical alternans was observed in whole hearts using extracellular (Hellerstein and Liebow, 1950) and intracellular (Downar *et al.*, 1977) recording techniques. In the latter case, even though one records the transmembrane potential of a single cell, it is impossible to say whether or not the beat to beat alternation in the morphology of the action potential seen in that cell is intrinsic to the cell or is due to an electrotonic injection of current from neighbouring cells that are coupled to the cell in question by low-resistance gap-junctional pathways. In the latter case, the alternans can be at least partly due to the fact that the stimulus current delivered to the cell is itself alternating. For example, in virtually all types of cardiac tissue, alternation of action potential morphology can be seen in the region of block when 2:1 block occurs (see Guevara, 1984, for references). Modelling work indicates that in one such instance alternation is indeed secondary to the fact that the flow of stimulus current into the region of block from regions distal to the site of block is itself alternating: the alternation is thus not intrinsic to the cells in question (Guevara, 1988, figure 3D).

In light of the above considerations, it seemed timely for us to investigate: (i) whether or not a single, isolated, quiescent ventricular cell could be made to display alternans; (ii) whether or not alternans could be seen in a mathematical model of a patch of isopotential membrane.

METHODS

Experimental

Single ventricular cells are isolated from rabbit hearts using standard techniques (Giles and van Ginneken, 1985; Giles and Imaizumi, 1988). The heart is removed from the animal, and the coronary arteries perfused with a heated (37°C), oxygenated, buffered salt solution flowing through the aorta in the retrograde direction. The heart is first perfused using a solution with a physiological Ca^{2+} concentration (2.2 mM) to wash out the blood from the coronary bed, then with a low-Ca^{2+} solution, and finally with a low-Ca^{2+} solution containing a mixture of the enzymes collagenase (Sigma, type 1A: 500 U/ml) and trypsin (Sigma, type 11: 249 u/ml). The low-Ca^{2+} solution is made by adding 15 μm Ca^{2+} to a nominally Ca^{2+}-free solution. The composition (mM) of the nominally zero [Ca^{2+}] solution is: NaCl 121, KCl 5.0, $MgCl_2$ 1.0, $NaHCO_3$ 15, Na_2HPO_4 1.0, Na-CH_2COOH 2.8, glucose 5.5. All solutions are gassed with carbogen (95% O_2–5% CO_2). Strips of tissue are then cut out of the right ventricle. These strips are cut into chunks, which are placed into a heated (37°C) bath containing low-Ca^{2+} solution sitting on a gyrating table rotating at 70 r.p.m.. This combined mechanical and chemical digestion breaks the chunks up, yielding a suspension of single cells.

The cells are then placed into a chamber sitting at room temperature (24–27°C) on the stage of an inverted microscope. The above isolation procedure produces quiescent, rod-shaped cells which are 'calcium-tolerant', i.e. which do not die when the experimental chamber is perfused with solution at a physiological Ca^{2+} concentration of 2.2 mM. A glass suction microelectrode is then positioned using a micromanipulator to come into contact with the membrane of a cell, whereupon an electrode–membrane seal of resistance 2–15 GΩ is formed upon application of slight suction. A suction pulse then disrupts the patch of membrane sealed off by the tip of the microelectrode. Since the electrolytic solution within the pipette (composition (mM): KCl 140, EGTA 5, $CaCl_2$ 1.54, $MgCl_2$ 1, Na_2ATP 5, HEPES 5, buffered to pH = 7.0 with KOH) is then contiguous with the intracellular fluid, the potential difference between the microelectrode and a second electrode placed in the bathing fluid surrounding the cell can then be measured. This potential difference is the transmembrane potential. A periodic train of current pulses of constant amplitude can then be delivered to the cell through the same microelectrode to stimulate the cell.

Numerical

We know of no published ionic models of the rabbit ventricular heart cell. For this reason, we investigate the model of Beeler and Reuter (1977) which describes ungulate ventricular fibres. There are five ionic currents in this model: the fast inward sodium current (I_{Na}), the slow inward calcium current (I_s), the time-dependent outward potassium plateau current (I_{x1}), and the time-independent potassium and sodium leakage currents (I_{K1}, $I_{Na,c}$). Numerical integration is carried out in single precision (approximately seven significant decimal digits) on a Hewlett-Packard minicomputer (model 1000F) using an efficient variable time-step Euler algorithm (Victorri *et al.*, 1985) previously used by us in a modelling study of phase-resetting in Purkinje fibre (Guevara and Shrier, 1987). The maximum change in the transmembrane potential ΔV allowed in iterating from time t to time $t + \Delta t$ is 0.4 mV. When a value of ΔV larger than this upper limit results, the integration time step Δt is successively halved and the calculations redone until ΔV is less than 0.4 mV. When ΔV is less than 0.2 mV, Δt is doubled for the following iteration. Under these conditions, the value of Δt ranges between 1 μs and 8.192 ms, and the voltage waveform is very close to that

appearing in the original description of the model (Beeler and Reuter, 1977). In advancing from time t to time $t+\Delta t$, the contribution of the transmembrane current to ΔV is calculated using the formula appearing in footnote 2 of Victorri *et al.* (1985). Since the time step is variable, it must be adjusted when current pulses are delivered so that the current starts and stops at exactly the right times. The time constants and asymptotic values of the various activation and inactivation variables (m, h, j, d, f, x_1) are stored in a table at steps of 0.2 mV and linear interpolation is used to extract out their values at any given voltage V. L'Hôpital's rule must be applied when necessary in calculating the rate constant α_m and the current I_{K_1}. Initial conditions on activation and inactivation variables were those appropriate to the membrane resting at $V=-84$ mV for an infinitely long time, and the initial condition on internal $[Ca^{2+}]$ was $[Ca^{2+}]=0.1$ μM. This voltage of -84 mV is close to the resting potential in the model.

RESULTS

Experimental

For the pulse amplitude (A) sufficiently low, one obtains only a subthreshold response to each stimulus of the pulse train. This 1:0 response can be seen at any value of t_s, the time between stimuli. For A sufficiently high at a given t_s, the stimulus is suprathreshold, and so one can obtain action potentials. In that case, for t_s sufficiently large, one sees a 1:1 rhythm, in which each stimulus produces an action potential (Fig. 1A). (An N:M rhythm is a periodic pattern consisting of repeating N:M cycles each containing N stimuli and M action potentials. The period of repetition of the waveform is thus Nt_s. The M action potentials will in general have M different morphologies.)

For A chosen correctly, as t_s is decreased, the 1:1 rhythm will be converted directly into a 2:2 rhythm, without any other pattern being seen. Figure 1B shows an example of a 2:2 rhythm, showing an alternans in the morphology of the action potential. While there is an alternation in many of the parameters that can be used to describe an action potential, the alternation is probably most striking in action potential amplitude and duration. As t_s is decreased further, the alternans becomes more marked, with the amplitude and duration of the smaller action potential decreasing. Eventually, the 2:2 rhythm converts into a 2:1 rhythm, with every second stimulus no longer producing an action potential (Fig. 1C).

There is both hysteresis and bistability present in the behaviour shown in Fig. 1. If t_s is first decreased and then increased so that the border between two patterns is traversed twice, the value of t_s at which the transition occurs is different, depending upon the direction of change of t_s. This 'frequency hysteresis' has a counterpart in 'amplitude hysteresis', in which the pattern

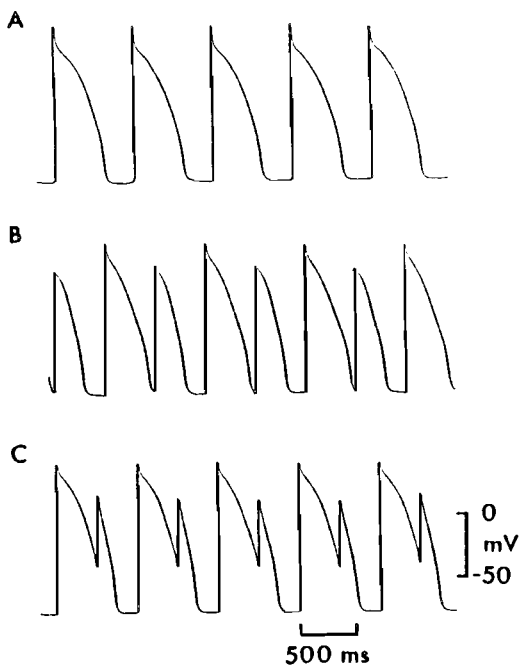

Fig. 1. Alternans in an isolated rabbit ventricular cell. The transmembrane potential is shown as a function of time. (A) 1:1 rhythm, $t_s=700$ ms. (B) 2:2 rhythm, $t_s=430$ ms. (C) 2:1 rhythm, $t_s=350$ ms. Pulse amplitude=1.0 nA, pulse duration=10 ms. Temperature=26°C. Sufficient time has been allowed to let transients pass. Stimulus artefact retouched.

seen at a particular amplitude at fixed t_s depends on whether A was increased or decreased to arrive at that value. If a well-timed extra stimulus pulse is added to the basic current pulse train, it is possible to convert a 2:1 rhythm into a 2:2 or a 1:1 rhythm. Injecting an extra stimulus or dropping one stimulus of the periodic drive train can also convert a 1:1 or 2:2 rhythm into a 2:1 rhythm. This illustrates that bistability is present, with the cell being capable of supporting two different periodic rhythms at a fixed combination of A and t_s.

Modelling

Figure 2A shows that, for t_s and A sufficiently large, 1:1 synchronization results. As t_s is decreased, the point is arrived at where one begins to see an alternation of action potential morphology. Figure 2B shows an example of such a 2:2 rhythm. As in the case of the experimental work, the alternation is

perhaps most obvious in action potential amplitude and duration, and becomes more marked as t_s is decreased. Eventually, as in the experimental work, a 2:1 rhythm results (Fig. 2C).

Assuming a typical apparent membrane surface area of 5200 μm^2 and an apparent specific capacitance of 1.4 $\mu F/cm^2$ for a single rabbit ventricular cell (Giles and Imaizumi, 1988), one can calculate that the current of 1 nA used in Fig. 1 corresponds to a true current density of about 14 $\mu A/cm^2$, provided that the true specific capacitance is 1 $\mu F/cm^2$ in the absence of infoldings in the cell membrane. For a 20 ms duration pulse, an equivalent effect would be produced at a current density of about 7 $\mu A/cm^2$, which is quite close to the 3 $\mu A/cm^2$ actually used in the model in Fig. 2. The current density employed in the model also compares well with experimental results in isopotential spontaneously beating (Guevara, 1984) and quiescent (Guevara *et al.*, 1984)

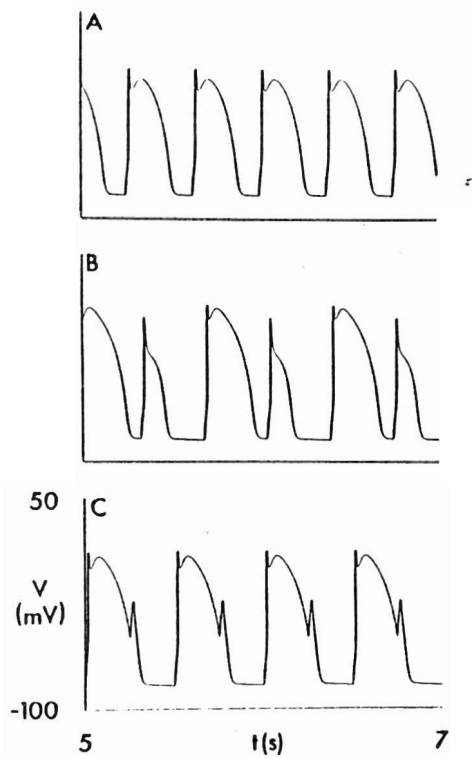

Fig. 2. Alternans in the Beeler–Reuter model. The transmembrane potential (V) is shown as a function of time (t). (A) 1:1 rhythm, t_s=375 ms. (B) 2:2 rhythm, t_s=355 ms. (C) 2:1 rhythm, t_s=250 ms. Pulse amplitude=3 $\mu A/cm^2$, pulse duration=20 ms. The first stimulus pulse is injected at t=0, and 5 s are allowed to let transients pass.

aggregates of embryonic chick ventricular cells, where a current density of about 6 $\mu A/cm^2$ is needed to produce alternans when current pulses of duration 20 ms are used (aggregate diameter $\simeq$ 100 μm, total surface area $\simeq 2.5 \times 10^{-3}$ cm^2, pulse amplitude $\simeq$ 16 nA).

DISCUSSION

Alternans in ventricular tissue

The trace shown in Fig. 1B answers more than a century later a question raised in the work of Gaskell (1882). It demonstrates that it is indeed possible to obtain intrinsic alternation in a single, quiescent, ventricular myocyte. However, it does not answer the question of what is happening in the left ventricle during electrical alternans. There are relatively few recordings of the transmembrane potential in that circumstance, either in the intact ventricle or in isolated pieces of ventricular muscle. In most such experiments, only one impalement is made, and so one does not know if all cells are alternating in a 2:2 rhythm. One experiment in which four simultaneous intracellular impalements were made during ischaemia indicates that a 1:1 pattern was present in the non-ischaemic myocardium, while 1:0, 2:1 and 2:2 patterns were present in the ischaemic area (Downar *et al.*, 1977, figure 10). In that case, the pattern of alternation that would be ascribed to the ventricle as a whole is due to the existence of an alternating rhythm in one subpopulation of cells as well as a 2:1 rhythm of block in yet another subpopulation. The existence of either one of these supopulations alone would theoretically be sufficient to generate an overall alternation in the ventricle. When both 2:2 and 2:1 rhythms occur together, it is impossible to decide which of these two rhythms would be the more important in generating alternation of the QRST complex of the surface electrocardiogram or mechanical alternans, since this would depend both on the relative sizes of the two subpopulations and also on the degree of alternation present in the 2:2 response. Since mechanical alternation is sometimes discordant with electrical alternation, with the larger action potential producing the weaker contraction (Spear and Moore, 1971), one might even imagine a situation in which an overall mechanical alternans might not be seen, provided that the balance between the 2:2 and 2:1 subpopulations was just right.

In the case when 1:1, 2:2 and 2:1 rhythms are seen in different areas of the ventricle, it is possible that the 2:1 pattern is due to block of propagation of the cardiac impulse, with the 2:2 pattern being seen in the region of block. The 2:2 pattern seen in this circumstance is due to decremental conduction and can be seen when 2:1 block occurs in tissue taken from virtually all areas of the heart (see Guevara, 1984, for references). The possibility then exists that the 2:2 pattern in the region of block is partly due to electrotonic

injection of current from the distal 2:1 region, in a manner recently demonstrated in modelling work on a one-dimensional strand of Purkinje fibre (Guevara, 1988). The question of whether or not it is possible to obtain a ventricle with a 2:2 pattern in all cells, or a 2:2 pattern in one circumscribed area and a 1:1 pattern everywhere else, remains open. This question can be answered only when techniques that allow recording of the transmembrane potential from many sites simultaneously (e.g. using potential-sensitive optical dyes) would be applied.

Ionic basis of alternans

The ionic basis of the response shown in Fig. 1B remains undetermined. However, the electrophysiological origin of the 2:2 rhythm is essentially due to the fact that, at the stimulation rates employed in Fig. 1, the duration of an action potential decreases monotonically with a decrease in recovery time since the immediately preceding action potential (Hauswirth *et al.*, 1972; Boyett and Jewell, 1978; Guevara *et al.*, 1984). Since in modelling work one can plot out not only the transmembrane potential, but also the various currents, activation variables and inactivation variables, it will be possible in future work to determine the ionic mechanisms producing the 2:2 response shown in Fig. 2.

Loss of 1:1 synchronization

We have shown above that the sequence of rhythms {1:1→2:2→2:1} can be seen as t_s is decreased (Fig. 1). However, this occurs only at a sufficiently high stimulus amplitude. We have also observed on several occasions in isolated cells a direct transition from a 1:1 to a 2:1 rhythm, with no evidence of the 2:2 rhythm, when t_s is changed in steps of 10 ms. (Perhaps the earliest definitive example of a direct {1:1→2:1} transition is to be found in the work of Mines (1913), who studied the atropinized ventricle of the frog.) In addition to this direct {1:1→2:1} sequence, one can also see Wenckebach-like rhythms between the 1:1 and 2:1 rhythms at t_s is decreased, provided that the stimulus amplitude is just suprathreshold and t_s is quite large (Guevara *et al.*, 1989a). The existence of these three qualitatively different ways in which 1:1 synchronization can be lost has been previously described in periodically stimulated, spontaneously beating, embryonic chick ventricular heart-cell aggregates with the {1:1→2:2} transition occurring at high stimulus amplitude, the {1:1→2:1} transition at intermediate amplitude, and Wenckebach rhythms at low amplitude (Guevara, 1984; Guevara *et al.*, 1989b).

Reduction to a one-dimensional map

We have shown previously that dynamics similar to that shown in Fig. 1 seen in periodically stimulated, quiescent chick ventricular aggregates can be reduced to the study of the iteration of a one-dimensional finite-difference equation or map (Guevara *et al.*, 1984). This map gives the ith action potential duration as a function of the immediately preceding one and is obtained from consideration of the curve describing how action potential duration is restored as a function of the recovery time since the immediately preceding action potential. In that case, it can be shown that alternans occurs when the slope of the map at the fixed point becomes more negative than -1, leading to a period-doubling bifurcation (Guevara *et al.*, 1984, figure 4). The sequence of rhythms predicted from the map is then {1:1→2:2→2:1}, which agrees with what is seen experimentally. A similar analysis should be possible in the case of Figs 1 and 2. The first analysis of electrical alternans formulated using an iterative technique seems to have been that of Nolasco and Dahlen (1968), while mechanical alternans has been similarly studied (Mahler and Rogel, 1970).

When the recovery of action potential duration is sufficiently rapid, all points on the map have a slope more positive than -1, and so a period-doubling bifurcation cannot occur. In that circumstance, one obtains a direct {1:1→2:1} transition. Note that in this instance, the transition is not due to a period-doubling bifurcation (cf. Chialvo and Jalife, 1987). The 2:1 rhythm corresponds to a period-1 orbit on the map, while the 2:2 rhythm seen if the map is sufficiently steep corresponds to a period-doubled period-2 orbit (Guevara *et al.*, 1984, figure 4). The transition {2:2→2:1} seen in a simple model of a limit-cycle oscillator (Guevara and Glass, 1982) and in experimental work on spontaneously beating heart-cell aggregates (Guevara, 1984, figure 5-16) is due to a change in rotation number.

As mentioned previously, when stimulating with large t_s ($\sim$1 s) and small A, Wenckebach rhythms can be observed at values of t_s intermediate to those at which 1:1 and 2:1 rhythms occur. At such low stimulation frequencies in the rabbit ventricle, the action potential duration shortens with increasing recovery time (Saxon and Safronova, 1982; Giles and Imaizumi, 1988) – it does not prolong as at the higher stimulation frequencies employed in Fig. 1. One thus obtains a different class of maps which have two branches, each of which has positive slope everywhere (Guevara *et al.*, 1989a). Thus, a period-doubling bifurcation cannot occur. These maps predict the existence of Wenckebach rhythms, and are qualitatively similar to a class of maps derived in recent work on Wenckebach rhythms in the human atrioventricular node (Shrier *et al.*, 1987). Wenckebach rhythms can also be seen in periodically stimulated, spontaneously beating, embryonic chick ventricular aggregates (Guevara *et al.*, 1988); in that case, one can sometimes reduce the dynamics

to iteration of a circle map whose slope is everywhere positive (e.g. Guevara, 1984, figure 5-4).

Alternans and ventricular arrhythmias

Electrical alternans has been described in many circumstances that are associated with ventricular fibrillation. These include coronary occlusion (Downar *et al.*, 1977; Adam *et al.*, 1984; see Guevara, 1984, for further references), abnormality of the sympathetic nervous system (Crampton, 1978), hypothermia (Adam *et al.*, 1984), and ventricular tachycardia (Sano *et al.*, 1958; Hogancamp *et al.*, 1959). In several of the above reports, intracellular impalements were made, and beat to beat alternation of the action potential morphology was recorded.

The close temporal connection between the phase of alternans and the phase of induction of malignant ventricular tachyarrhythmias might be entirely circumstantial. We think not. It has been suggested that the alternans seen in this instance and in other circumstances is due to a period-doubling bifurcation (Guevara *et al.*, 1981, 1984, 1989b; Guevara and Glass, 1982; Goldberger *et al.*, 1984; Guevara, 1984; Ritzenberg *et al.*, 1984; Adam *et al.*, 1984), and the implication has been made that this (first) bifurcation might be the initial part of a cascade of such bifurcations that would eventually result in chaos, i.e. fibrillation (Adam *et al.*, 1984).

However, at the present time, there is no experimental evidence for the existence of higher-order period doublings in the ischaemic heart. While period-4 rhythms have been identified on many occasions in experimental and modelling work on cardiac muscle, one must exercise caution in claiming that they result from two successive period-doubling bifurcations. There are at least four instances on record where the period-4 rhythm is clearly not the result of two such bifurcations (Guevara, 1984, figure 4-8C, figure 4-26C; Chialvo and Jalife, 1987, figure 2B (third trace); Guevara, 1988, figure 3B).

In our work with isolated rabbit ventricular cells, the rhythms 1:1, 2:2, 2:1, 4:2, 3:1 and 6:2, as well as apparently non-periodic rhythms, are often seen as t_s is decreased in steps of 10 ms. Note that while three period-doubling bifurcations occur {1:1→2:2}, {2:1→4:2} and {3:1→6:2}, they do not form part of a period-doubling cascade. For example, we have not seen the 2:2 rhythm period-double to a period-quadrupled 4:4 rhythm, as occurs with stimulation at low frequencies in spontaneously beating aggregates of embryonic chick ventricular cells (Guevara *et al.*, 1981).

In the case of induction of ventricular arrhythmias, we feel that one should leave open the possibility that there might be a direct transition from a period-doubled 2:2 or 4:2 pattern to a state of ventricular tachycardia or fibrillation. With regard to the latter eventuality, there are apparently mathematical schemes in which it is possible to generate chaotic behaviour following a single bifurcation (Tresser *et al.*, 1980). Should the first qualitative change occurring in the ischaemic ventricle be a transition from a 1:1 response in all cells to a 2:2 response in some subpopulation within the ischaemic zone, there would be still one action potential for each supraventricular stimulus in all cells of the ventricle, and the degree of spatient inhomogeneity would not be too pronounced. However, should some of the cells showing a 2:2 response proceed to the stage of showing a 2:1 response as the ischaemia becomes more profound, there would then be a greater degree of spatial inhomogeneity, with some areas demonstrating action potentials only on alternate beats. This might predispose the ventricle to formation of re-entrant rhythms.

In summary, while we have shown above that alternans can be generated by a single cell, it will take much more work to put this finding into perspective once one realizes that cells in the heart talk to one another.

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Cell to Cell Signalling: From Experiments to Theoretical Models

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