THE INTERVAL-STRENGTH RELATIONSHIP IN MAMMALIAN ATRIUM: A CALCIUM EXCHANGE MODEL

I. THEORY

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ABSTRACT A model is developed for predicting the interval-strength relationship in mammalian atrium. The postulates underlying the model relate the intracellular and transmembrane calcium fluxes to changes in contractility. The predictions of the model agree qualitatively with the behavior of atrium for the following patterns of stimulation: (*a*) constant interval between stimuli, (*b*) a rest, or period with no stimuli, after the attainment of a steady-state force level, (*c*) a sudden change in the interval between stimuli, and (*d*) paired pulse stimulation. The effects of varying several parameters of the model on both the contraction staircases, after a rested-state contraction, and the steady-state interval-strength relationship are examined. Additional considerations are made: (*a*) estimates are made of the tissue calcium content available for contraction; (*b*) the physical meaning of the rested-state contraction is discussed; and (*c*) estimates are made of the proportionality constant between the maximum value of the contractile tension and the amount of calcium released before a contraction.

INTRODUCTION

The interval-strength relationship encompasses all influences which the intervals between beats have on the strength of contraction in heart muscle (1). We will restrict our attention to isometric contractions of muscles which do not contract unless artificially stimulated, although some of our conclusions may apply also to automatic heart muscle, i.e., muscle which beats spontaneously. We further restrict our attention to stimulus frequencies which produce single contractions with each stimulus.

The interval-strength relationship includes the influences of such stimulus patterns as (*a*) constant period or interval between stimulations, (*b*) sudden changes in the period of stimulation, (*c*) a rest after a regular train of contractions and fol-

lowed by another regular train of contractions, (*d*) premature stimulation, (*e*) extrasystoles, and (*f*) "paired pulses."

If periodic stimulation (*a* above) is maintained, the muscle eventually attains a steady state during which the contractile force does not change measurably from beat to beat. The magnitude of this force depends strongly on the interval between beats. Other contraction patterns, e.g. *b–f* above, are accompanied by contractile "staircases" or "treppes". A staircase is said to occur whenever the contractile force changes from beat to beat; if the force increases or decreases progressively, the staircase is positive or negative, respectively.

The effects which contraction patterns such as *a–f* above have on the contractile strength of heart muscle are often complex. They vary not only among species and among animals of the same species, but also between the atrial and ventricular muscle of the same species (1–3). Several investigators have developed frameworks for describing some of these contractile changes. Bravený and Kruta (4) have defined two factors which determine the force of contraction: "potentiation" and "restitution". Blinks and Koch-Weser (1–3) have identified three properties of heart muscle: the rested-state contraction, positive inotropic effect of activation (PIEA), and negative inotropic effect of activation (NIEA). NIEA and PIEA are produced with every beat and decay between beats; the contractile force of a beat equals the rested-state contraction plus the PIEA, minus the NIEA in the muscle at the time of activation. Orkand (5) has defined a quantity called "facilitation" for predicting the contraction staircase in frog ventricle strips stimulated at different frequencies and under different conditions. Facilitation, like PIEA, is produced with every beat and decays between beats; the contractile strength of a given beat depends on the linear sum of the facilitation remaining from previous beats. Posner and Berman (6) gave mathematical analyses of the recovery of contraction after the rested-state contraction and the cumulation and disappearance of potentiation in rat ventricle strips. Bautovich et al. (7), Johnson et al. (8), and Johnson and Kuohung (9) used differential equations to describe the relationship between the contractile force of rabbit papillary muscle and the frequency and pattern of stimulation of the muscle.

The reports listed above used the maximum value of the contractile force or the maximum value of the time derivative of the contractile force as indices of contractile changes. Such a treatment may, for some heart muscle, oversimplify the descriptions of interval-dependent contractile changes. Blinks and Koch-Weser (1) have examined the effect on the time course of single contractions which the interval between contractions has on the following types of heart muscle: For cat papillary muscle, shortening the interval between beats from 300 to 0.25 sec increases the rate of both rise and decline of tension, and decreases the time required for the tension to peak. For tortoise ventricle, Blinks and Koch-Weser showed that shortening the interval between beats from 5 to 1 sec decreases the time required for the tension to peak, but has little influence on the rate of rise or decline of ten-

sion. The time required for the tension to peak for guinea pig atrium, on the other hand, does not change significantly as the interval between beats is shortened from 300 to 0.32 sec; the shape of the tension-time curve changes by only a scale factor over this wide range of intervals, and hence, the maximum value of the contractile force should provide an adequate index for measuring changes in contractility. For this reason, we have chosen to examine the interval-strength relationship in guinea pig atrium. Because the qualitative features of the interval-strength relationships of most mammalian atria resemble those of the guinea pig (1), we expect that the conclusions obtained from a study of this tissue will probably apply, at least qualitatively, to other mammalian atrial muscle. We shall now discuss briefly those aspects of the role of calcium in muscle contraction which are pertinent to the subsequent discussion.

It is generally accepted that $\{Ca^{+2}\}_i$, the intracellular concentration of free calcium after an electrical stimulation, determines the tension development of a muscle (10, 11). We therefore assume that changes in the contractile strength of heart muscle may reflect changes in $\{Ca^{+2}\}_i$ after stimulations. In order to explore this possibility, we have developed a model for predicting changes in the contractile strength of cardiac muscle based on a kinetic analysis of calcium movements between contractions.

Calcium Movement and Contractility

An action potential is accompanied by a transient increase in $\{Ca^{+2}\}_i$ from below the activation threshold calcium concentration, about 5×10^{-7} M, to values of the order of 10^{-5} M (12). This increase results from transmembrane calcium ion influx during the action potential, or the release of calcium from one or more intracellular calcium storage compartments. The influx per beat is of the order of 10^{-9} moles Ca/g tissue per beat (13–17) or less (18), and therefore probably cannot, by itself, raise $\{Ca^{+2}\}_i$ to the activation threshold. The cumulative effect of the calcium influx accompanying several action potentials can, however, contribute significantly to the $\{Ca^{+2}\}_i$ for subsequent contractions if this calcium is incorporated into calcium storage compartments. Considerable evidence indicates the existence of calcium storage compartments in skeletal muscle (19, 20). In cardiac muscle (11, 13–15), there appears to be at least one calcium storage compartment which controls the $\{Ca^{+2}\}_i$, and, hence, the contractile response of cardiac muscle. The most likely cellular structure for such storage is the sarcoplasmic reticulum. Based partly on the estimated calcium binding capacity of the sarcoplasmic reticulum and on the close proximity of portions of the sarcotubular system to the contractile mechanism, Langer and his associates (14, 15) have suggested that this system is the compartment that binds the calcium represented kinetically by his "phase 2" of the calcium washout. The phase 2 calcium level and the systolic tension seem to be intimately related because the increase in contractile strength after an increase

in contraction rate, and the decrease in contractile strength when the muscle was perfused with 0 Ca^{+2} perfusate, were accompanied by parallel increases and decreases in the calcium level of the compartment associated with phase 2. Langer and his coworkers advanced the hypothesis that the increase in the contractile strength is controlled indirectly by the sodium exchange in the tissue. For example, an increase in contraction frequency produces a concomitant increase in action potential sodium influx. While the active sodium pump slowly increases its pumping rate to handle the increased load, a transient increase in intracellular sodium can occur. The added sodium competes with and displaces calcium in the phase 2 region for binding sites. The displaced calcium causes a transient rise in contractile strength. A transient gain in phase 2 calcium after a frequency change also occurs. Sonnenblick and Stam (21) point out that a lag in an active pump which controls the calcium efflux can explain this calcium gain without recourse to a lag in the sodium pump.

MODEL

The calcium flux data in the literature (16–18) and the evidence for the dependence of the contractile strength on calcium suggest that a model based partly on intracellular and transmembrane calcium movement could account for some of the contractile changes observed in cardiac muscle. The model we propose incorporates the action potential calcium influx, rest calcium influx, active calcium efflux, the intracellular calcium movements both before a contraction and between contractions, and certain contractile data. Such a model necessarily simplifies the mechanisms proposed. For simplicity, we assume that a single compartment, n_1, releases the calcium used by the contractile mechanism. We assume that a fraction f of the calcium in n_1 is released when the muscle is stimulated. This fraction may depend on the frequency of contraction, but is assumed to be constant at a given frequency. After the release of calcium, another compartment, n_2, actively sequesters the calcium. This second compartment then returns the calcium—perhaps actively—to the first compartment. We will make no assumptions about the location of the compartments since neither the location nor identity of the compartments is essential to the argument which follows. The calcium

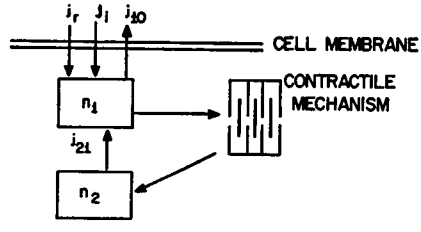


FIGURE 1 A schematic diagram of the model. The arrows represent fluxes of calcium, or, in the case of J_i, the time integral of the action potential calcium flux over the duration of the action potential. n_1 and n_2 are two calcium storage compartments. The strength of a contraction is proportional to the amount of calcium released from n_1 to the contractile mechanism; this amount is proportional to the calcium content in n_1 at the moment of stimulation. After a contraction, n_2 acquires the calcium. Between beats, calcium is transferred from n_1 to n_2.

levels of n_1 and n_2, $n_1(t)$ and $n_2(t)$, respectively, change rapidly when the muscle is stimulated; the first compartment releases fn_1 moles of calcium after the stimulation and n_2 increases by the same amount during relaxation; between beats, calcium is transferred from n_2 to n_1. An amount of calcium influx, J_i, enters the cell during each action potential. This calcium is assumed to add onto n_1 and contribute to subsequent contractions. In addition, we assume that a constant passive calcium influx, j_r, also contributes to n_1. An active calcium pump appears to maintain the intracellular level (22). We have assumed that calcium is pumped out of the cell from n_1. The two calcium exchange cycles—one intracellular and one transmembrane exchange—are illustrated schematically in Fig. 1.

ANALYSIS

j_{10} and j_{21} in Fig. 1 are assumed to be proportional to the compartments from which they originate; thus $j_{10} = an_1$, and $j_{21} = bn_2$, where a and b are constants. The differential equations for the calcium levels of the two compartments n_1 and n_2 are

$$\frac{dn_1}{dt} = -an_1 + bn_2 + j_r, \tag{1}$$

and

$$\frac{dn_2}{dt} = -bn_2. \tag{2}$$

The effects of the calcium input per action potential, J_i, is considered below in the initial conditions of n_1 (equation 6). The solutions of equations 1 and 2 are

$$n_2(t) = Be^{-bt}, \tag{3}$$

$$n_1(t) = Ae^{-at} - \frac{bB}{b-a}e^{-bt} + \frac{j_r}{a}, \tag{4'}$$

where A and B depend on n_{10} and n_{20}, the values of n_1 and n_2 immediately after calcium is released from n_1. We will consider only those systems for which $b \gg a$; the equation for $n_1(t)$ becomes

$$n_1(t) = Ae^{-at} - Be^{-bt} + [j_r/a] \tag{4}$$

in this approximation. Equation 4 rather than equation 4′ will be used to describe the evolution of $n_1(t)$. According to the procedure adopted here, time is reset after each release so that n_1 and n_2 evolve from $t = 0$ for every contraction. If the muscle is stimulated at $t = T$ after a contraction, a fraction f of calcium in n_1 is released to participate in the contractile process. The amount of calcium in n_1 after the release is n_{10} for the next contraction.

$$n_{10} = (1 - f)n_1(T). \tag{5}$$

We have assumed that J_i adds immediately to n_1, so that the initial value of n_1,

$n_1(0)$, for the next contraction cycle is given by $n_{10} + J_i$ (where n_{10} is given by equation 5). This value must also satisfy equation 4 with $t = 0$, i.e.,

$$n_{10} + J_i = A - B + [j_r/a]. \tag{6}$$

Using equation 3, the initial value of n_2 is

$$n_{20} = B. \tag{7}$$

Solving for A and B,

$$A = n_{10} + n_{20} + J_i - [j_r/a], \tag{8}$$

and

$$B = n_{20}, \tag{9}$$

and equations 4 and 3 become

$$n_1(t) = (n_{10} + n_{20} + J_i - [j_r/a])e^{-at} - n_{20}e^{-bt} + [j_r/a], \tag{10}$$

and

$$n_2(t) = n_{20}e^{-bt}. \tag{11}$$

The initial value of n_2 depends on the rapidity with which the calcium used for a contraction becomes available for transport from n_2 to n_1. Of several possible initial conditions two mathematically tractable ones are presented: (*a*) The calcium used in a contraction becomes available for transport immediately after it is sequestered by n_2. In this case, we may approximate the initial condition n_{20} for the second contraction by

$$n_{20} = n_2(T) + fn_1(T), \tag{12}$$

where quantities on the right-hand side of equation 12 refer to values for the first contraction. (*b*) All the calcium used in a contraction becomes available for transport from n_2 to n_1 after a time delay (9). Other hypotheses about the nature of n_{20} are possible, but all are conjectural because the intracellular calcium fluxes cannot be accurately determined at this time. We therefore believe it best to make one or two arbitrary hypotheses about the initial conditions of n_2 and allow the possibility of later reanalysis of the data if new information reveals the mechanisms and time courses of calcium sequestration and transfer from n_2 to n_1. The consequences of the two hypotheses differ significantly from one another only when $n_1(T)$ changes very rapidly from contraction to contraction. We have arbitrarily assumed the hypotheses described in *a* above for the rest of this discussion. We have assumed that F, the maximum value of the developed tension during a contraction, is pro-

portional to $fn_1(T)$, the amount of calcium released by n_1 before a contraction:

$$F = Qfn_1(T), \tag{13}$$

where Q is a proportionality constant. Note from equation 10 that if the muscle is left unstimulated, n_1 attains a steady-state level of j_r/a which is independent of the initial conditions and previous contractile history of the muscle. We have, therefore, used this quantity to normalize both n_1 and n_2 in equations 10 and 11:

$$\text{Let } x = n_1/(j_r/a),$$

$$y = n_2/(j_r/a), \text{ and}$$

$$z = J_i/(j_r/a);$$

then

$$x(t) = (x_o + y_o + z - 1)e^{-at} - y_o e^{-bt} + 1, \tag{14}$$

and

$$y(t) = y_o e^{-bt}. \tag{15}$$

Effect of a Rest

According to equation 15, y decays exponentially after a contraction. The decay constant b is typically about 0.5 sec^{-1} in the guinea pig atrium (23) and hence y becomes negligible after about 10 sec. Equation 14 predicts that x increases at the expense of y during this time period, if no contraction takes place. For longer time periods, x declines exponentially to its final steady-state value of 1. The decay constant a is about 5×10^{-3} sec^{-1} in guinea pig left atria (23). If the muscle is stimulated to contract T_i sec after the previous contraction, a fraction f of calcium in n_1 is released to the contractile elements. According to equation 13, $F_1 = Qfn_1(T_i)$, where F_1 is the first contraction after the rest period T_i ; also

$$F_1 = \left\{\frac{Qfj_r}{a}\right\} x_1(T_i). \tag{16}$$

For very long rest periods, F is the *rested-state contraction*, F_R :

$$F_R = \frac{Qfj_r}{a}. \tag{17}$$

From equations 16 and 17,

$$x_1(T_i) = \frac{F_1 a}{Qfj_r} = \frac{F_1}{F_R}. \tag{18}$$

Equation 18 is valid for all T_i if, as we have assumed, Q, f, j_r , and a are independent

of T_i. The validity of this assumption is difficult to test, although some of the equations derived below may be used to that end.

Equations 14 and 15 predict the evolution of x and y between beats. x_o, y_o, $x(T)$, and F change from beat to beat during a staircase, but, if a muscle is stimulated at regular intervals, T, these quantities eventually attain steady-state values.

A simple experiment for determining x_o, y_o, a, b, and f for different stimulus intervals T is as follows. The muscle is stimulated at regular intervals until the peak tension F developed in each contraction reaches a steady-state value F_{ss}. Stimulation is then interrupted for various time intervals T_i, allowing sufficient time between interruptions for the peak tension to return to F_{ss}. The first, second, $\cdots$, mth contractions after the interruption were designated F_1, F_2, $\cdots$, F_m, respectively. The normalized values of F_1, F_2, $\cdots$, F_m are x_1, x_2, $\cdots$, x_m, respectively, where $x_m = F_m/F_R$ as before. Fig. 2 illustrates the x_1 data for a tissue along with the curve fitted according to equations 14 and 18 (23). Using equation 14 and recalling that $b \gg a$, for $T_i \gtrsim 10/b$, $x(T_i)$ is given essentially by

$$x(T_i) = (x_o + y_o + z - 1)e^{-aT_i} + 1; \tag{19}$$

if $\log \{x(T_i) - 1\}$ is plotted vs. T_i for large values of T_i and fit by a straight line, the slope of the line will be $-a$ and the intercept at $T_i = 0$ will be $x_o + y_o + z - 1$.

Similarly, if $\log \{(x_o + y_o + z - 1)e^{-aT_i} + 1 - x(T_i)\}$ is plotted for small values of T_i and fit by a straight line, the slope of the line will be $-b$ and the intercept at

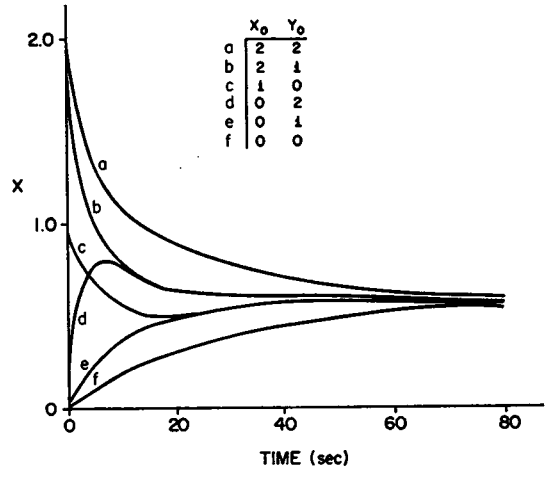


FIGURE 2 Effect of a rest. A muscle stimulated at constant intervals acquires a steady-state contractile force. After a steady-state contraction, the muscle is allowed to rest for a time T_i before stimulation is resumed. The first contraction normalized with respect to the rested-state contraction is x_1. x_1 is plotted here for a guinea pig atrium stimulated at T = 2 sec (23). We have included the steady-state x and an extrasystole. The equation of the curve is $x_1(T_i) = 1 + 0.16 \exp(-0.005T_i) - 0.08 \exp(-0.46T_i)$.

$T_i = 0$ will be y_o. The quantity $z = (J_i a/j_r)$ can be calculated using J_i and j_r obtained from ^{45}Ca tracer data (e.g., 16–18) and a determined in the manner described above. Thus it is possible to determine x_o and y_o. The fraction f can be found by a normalized version of equation 5:

$$x_o = (1 - f)x(T), \tag{20}$$

where $x(T)$ is given by equation 14 with $t = T$. This method of analysis is particularly simple because the initial conditions for $x_1(T_i)$ are the same as those for the steady-state contractions.

Staircase after a Rest Period

The normalized strength of the second contraction after a rest interval T_i can be predicted from the data found in the above experiment. The values of x and y immediately after the first contraction are x_{o2} and y_{o2}:

$$x_{o2} = (1 - f)x_1(T_i), \tag{21}$$

and

$$y_{o2} = y_o e^{-bT_i} + fx_1(T_i), \tag{22}$$

where we have assumed, as above, that the calcium released to the contractile elements adds immediately to y. Substituting x_{o2} and y_{o2} in equations 21 and 22 for x_o and y_o in equations 14 and 15, with $t = T_i$, to find $x_2(T)$,

$$x_2(T) = \{(1 - f)x_1(T_i) + y_o e^{-bT_i} + fx_1(T_i) + z - 1\}e^{-aT} - \{y_o e^{-bT_i} + fx_1(T_i)\}e^{-bT} + 1. \tag{23}$$

Rearranging,

$$x_2(T_i) = \{x_1(T_i) + y_o e^{-bT_i} - 1\}e^{-aT} - y_o e^{-b(T_i+T)} + 1 - fx_1(T_i)e^{-bT} + ze^{-aT}, \tag{24}$$

but from equations 14 and 15,

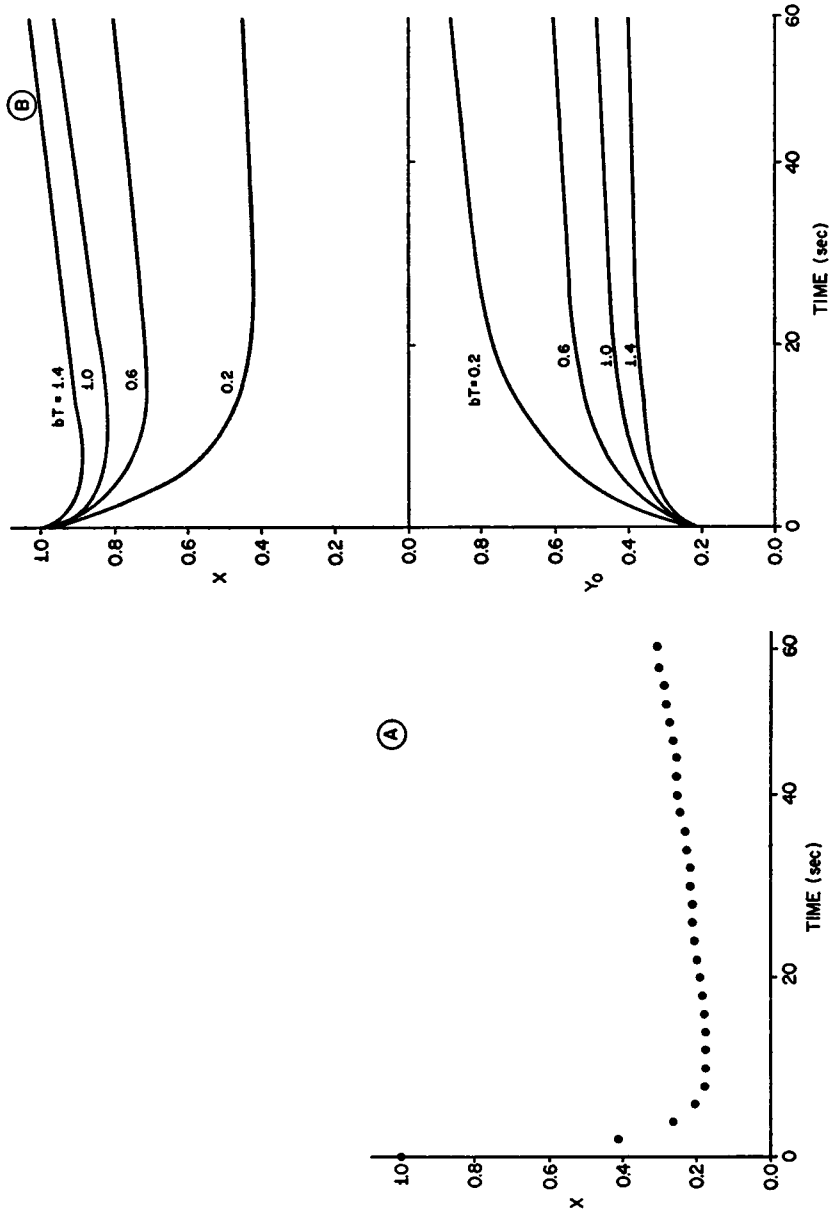
$$x_1(T_i) + y_o(T_i) - 1 = (x_o + y_o + z - 1)e^{-aT_i}, \tag{25}$$

and thus,

$$x_2(T) = (x_o + y_o + z - 1)e^{-a(T_i+T)} - y_o e^{-b(T_i+T)} + 1 - fx_1 e^{-bT} + ze^{-aT}; \tag{26}$$

or, using equation 14 with $t = T_i + T$,

$$x_2(T) = x_1(T_i + T) - x_1(T_i)fe^{-bT} + ze^{-aT}. \tag{27}$$


B
bT = 1.4
1.0
0.6
0.2
X
bT=0.2
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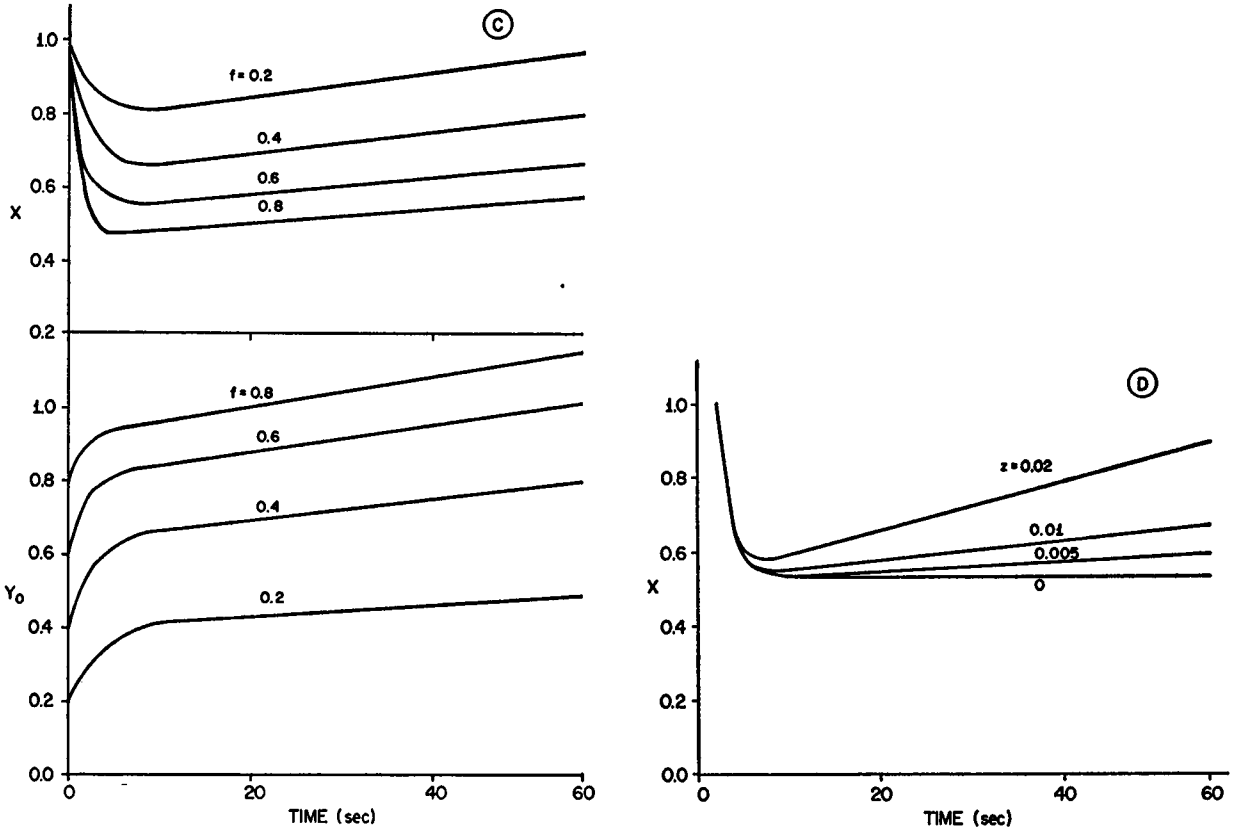

FIGURE 3 Staircase following a rested-state contraction. All curves are envelopes (i.e., the curve passing through successive maxima of x) of either normalized contractile force, x, or y_o just after a contraction. The abscissa is the time after the rested-state contraction. 3 A shows the staircase of a guinea pig atrium stimulated at 2-sec intervals. 3 B–D show several staircases generated by the model. In B, the effect of varying bT is illustrated. Other parameters are $aT = 0.01, f = 0.2, z = 0.01$. In C, f is varied. Other parameters are $aT = 0.01, bT = 1.0, z = 0.01$. In D, z is varied. Other parameters are $aT = 0.01$, $bT = 1.0, f = 0.6$.

Equation 27 can be viewed either as a method of predicting x_2 from values of x_1, or as an experimental technique for determining fe^{-bT}. Letting $fe^{-bT} = w$,

$$w = \frac{x_1^{+} - x_2 + ze^{-aT}}{x_1}, \tag{28}$$

where $x_1 = x_1(T_i)$, $x_1^{+} = x_1(T_i + T)$ and $x_2 = x_2(T_i)$.

The initial values of x and y for the third contraction are similarly,

$$x_{o3} = (1 - f)x_2, \tag{29}$$

$$y_{o3} = y_{o2}e^{-bT} + fx_2. \tag{30}$$

Substituting x_{o3} and y_{o3} in equations 29 and 30 for x_o and y_o in equations 14 and 15, exactly as before, and using equations 27 and 14 for x_2, and x_1^{+},

$$x_3(T) = x_1^{++} - wx_1^{+} - x_1(we^{-bT} - w^2) + ze^{-aT}(1 + e^{-aT} - w), \tag{31}$$

where $x^{++} = x_1(T_i + 2T)$.

Obviously the same process can be repeated to generate the fourth and subsequent contractions, but it is not necessary to solve the equations for $x_m(T)$. A computer or calculator can be programmed to generate as many contractions in the staircase as desired. Fig. 3 shows predicted and experimental staircases. Fig. 3 also illustrates the effect which varying certain parameters in this model has on the staircases for which the first contractions are rested-state contractions. The contractile strength returns to a steady-state level $x_{ss}(T)$, but clearly the details of the return process and the value of $x_{ss}(T)$ depend on the choice of parameter. Equations 27 and 31 can be solved for b and f. Solving for e^{-bT} in equation 31,

$$e^{-bT} = \frac{x_1^{++} - x_1^{+}w + x_1w^2 - x_3 + ze^{-aT}(1 + e^{-aT} - w)}{wx_1}, \tag{32}$$

then

$$b = \frac{1}{T}\ln\left\{\frac{wx_1}{x_1^{++} - x_1^{+}w + x_1w^2 - x_3 + ze^{-aT}(1 + e^{-aT} - w)}\right\}; \tag{33}$$

from the definition $w = fe^{-bT}$, it follows that

$$f = \frac{w^2x_1}{x_1^{++} - x_1^{+}w + x_1w^2 - x_3 + ze^{-aT}(1 + e^{-aT} - w)}. \tag{34}$$

These parameters can be used in predicting the rest of the staircase. The equations related to this model predict the qualitative features of this staircase for mammalian atrium (1, 3). Fig. 3 shows that the quantitative features of the predictions depend strongly on the choice of parameters and initial conditions in equations 14 and 15.

Frequency Changes

The experimental data, i.e., the peak tension of successive contractions in the staircase after a rest period T_i, represent one of the simplest experiments in which the pattern of stimulation departs from periodic stimulation. Another simple experiment is a step change in frequency. A muscle stimulated at regular intervals T_1 attains a steady-state $x_{ss}(T_1)$, then the interval is changed suddenly to T_2 (either $T_2 > T_1$ or $T_2 < T_1$). A contraction staircase follows and the muscle eventually attains a new steady-state level $x_{ss}(T_2)$. The mathematical treatment of the staircase after a frequency change closely resembles that of the staircase after a rest period T_i. The first contraction, x_1, occurs T_2 sec after the least steady-state contraction at T_1. x_1 is

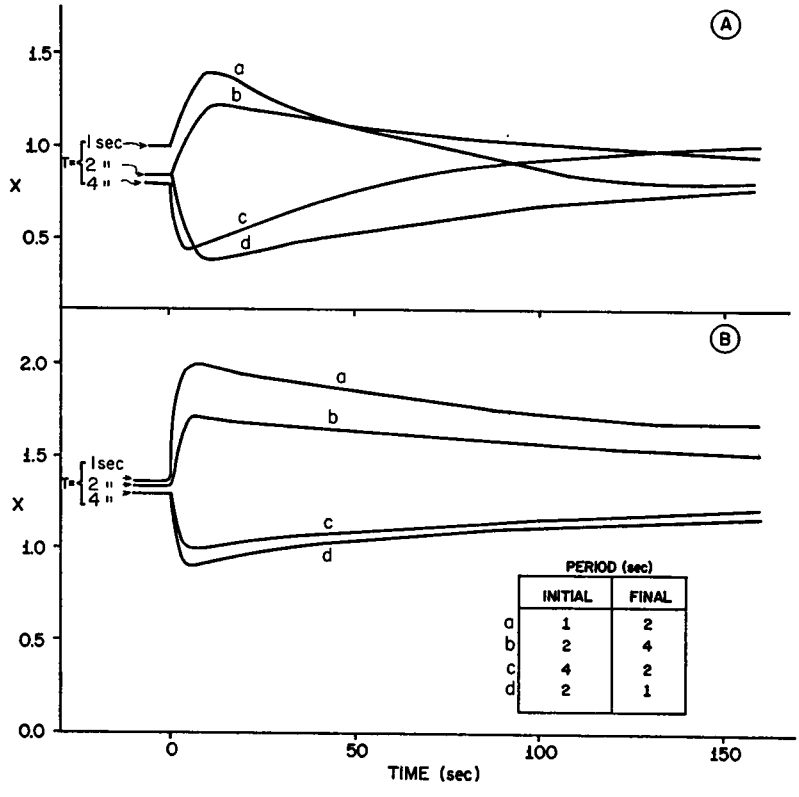

FIGURE 4 The effect of changing the interval between stimuli. The abscissa is the time after T, the interval between contractions, is changed. The ordinate is x, the normalized value of contractions. The horizontal arrows to the right of zero time represent the steady-state x before the change and the curves are the envelopes of x (i.e., the curve passing through successive maxima of x) as the contractile strength changes from one steady value to another. The behavior of a guinea pig atrium is shown in 4 A (Hollander, unpublished data). Fig. 4 B shows the results for the model undergoing the same stimulus patterns. The parameters of the model are $a = 0.005$ sec^{-1}, $b = 0.4$ sec^{-1}, $f = 0.6$, and $z = 0.01$. The initial and final periods for the curves in both 4 A and B are given in the table in 4 B.

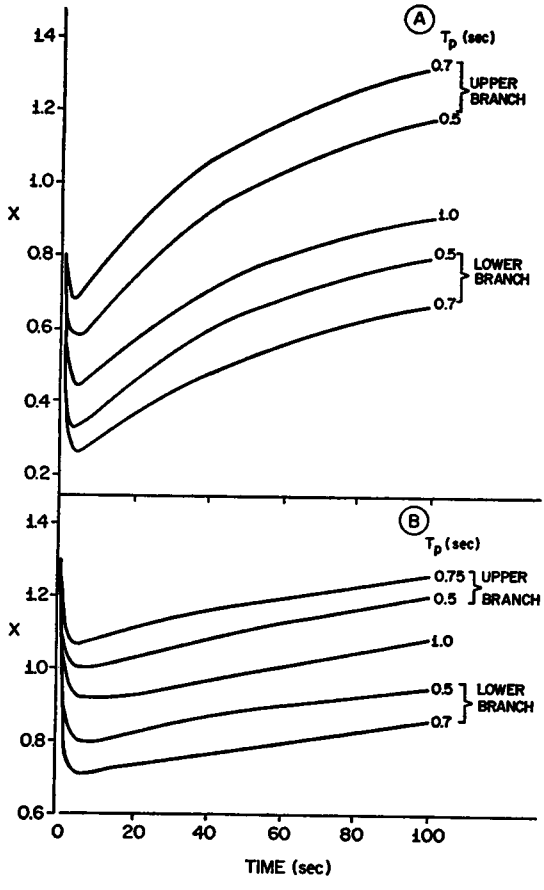

FIGURE 5

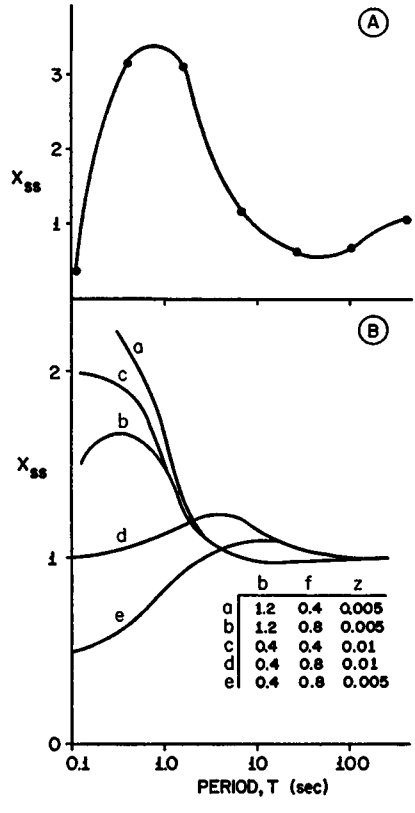

FIGURE 6

FIGURE 5 Paired stimulation. Paired stimuli were introduced in the manner described in the text. The curves are envelopes (i.e., the curve passing through successive maxima of x) of the branches described in the text. Figs. 5 A and B show the behaviors of a guinea pig atrium (Hollander, unpublished data) and the model, respectively. The model parameters are $a = 0.005$ sec^{-1}, $b = 0.4$ sec^{-1}, and $f = 0.6$. Both the atrium and the model were stimulated at 2-sec intervals before the onset of paired stimulation. At zero time the arrows indicate the steady-state values of x before the paired stimulation was introduced. FIGURE 6 The steady-state interval-strength relationship for (A) guinea pig left atria (Hollander, unpublished data), and (B) the model presented in this paper. The parameters for the curves are given in the inset; $a = 0.005$ sec^{-1} for all the curves in 6 B.

given by equation 14 where $t = T_2$. x_2 is given by a modified form of equation 27:

$$x_2(T_2) = x_1(2T_2) - fx_1(T_2)e^{-bT_2} + ze^{-aT_2}. \quad (35)$$

x_3 is given by a similar modification of equation 31. Fig. 4 shows the computed values for successive contractions after an increase and a decrease in the interval between stimuli together with the corresponding experimental results from a real

muscle. No effort has been made to fit the behavior of the model exactly to that of the muscle.

Paired Stimulation

In this study, paired stimuli were introduced in the following manner: The muscle was stimulated by a train of regularly spaced pulses until it attained a steady-state $x_{ss}(T)$. A second train of stimulating pulses with the same period was then introduced; the pulses of the second train were delayed by a time delay $T_p(<T)$ with respect to those of the first train. Thus intervals between pulses alternated between T_p and $T - T_p$.

The value reached by the theoretical quantity x just before a contraction alternates between two levels so that the envelope of these values forms two "branches" if $T_p \neq \frac{1}{2}T$. As in Fig. 4, no effort has been made to fit the behavior of the model exactly to that of the muscle. To be specific, suppose that $T_p < \frac{1}{2}T$; then x for contractions preceded by intervals equal to T_p are smaller than x for contractions preceded by intervals equal to $T - T_p$ because, in the latter case, the muscle has a longer time to recover from the previous contraction than in the former case. Thus x values of the contractions form two branches: those for beats preceded by intervals T_p and those for beats preceded by intervals $T - T_p$. Fig. 5 demonstrates the branching for several values of T_p, and shows the response of the model to paired stimulation. The two branches attain steady-state values of x, and when the second train is deleted, the contraction returns to its steady-state $x_{ss}(T)$.

STEADY-STATE INTERVAL-STRENGTH RELATIONSHIP

As we stated earlier, a muscle stimulated periodically eventually attains a steady-state $x_{ss}(T)$. Our model exhibits similar behavior. The approach to $x_{ss}(T)$ was shown to depend on both the initial values of x and y and the parameters a, b, f, z, and T. The final value, on the other hand, was shown to be independent of the initial values of x and y. Using equations 13–15, an equation will now be derived which predicts the steady-state interval-strength relationship for the model.

During steady-state contractions, y_o is the same from beat to beat. Using equations 15 and 22

$$y_o = \frac{f x_{ss}(T)}{1 - e^{-bT}}. \tag{36}$$

Substituting equations 36 and 21 into equation 14 evaluated at $t = T$, then solving for $x_{ss}(T)$,

$$x_{ss}(T) = \frac{(1 + z)/(e^{aT} - 1)}{(1 + f)/(e^{bT} - 1)}, \tag{37}$$

and hence

$$F_{ss}(T) = \frac{Qfj_r}{a} \frac{(1 + z)/(e^{aT} - 1)}{(1 + f)/(e^{bT} - 1)}. \quad (38)$$

Equation 38 allows for the possibility that f may be a function of T.

Equation 38 gives the steady-state contractile force as a function of the interval between beats. Note first that the limits of x_{ss} and F_{ss} for large T are

$$\lim_{T \to \infty} x_{ss} = 1, \quad (39)$$

and

$$\lim_{T \to \infty} F_{ss} = Qfj_r/a. \quad (40)$$

Formally, the limit of F_{ss} for small t can be found by applying L'Hospital's rule (e.g., 22) to equation 38:

$$\lim_{T \to 0} F_{ss} = \frac{Qzfj_r}{a^2}. \quad (41)$$

This equation is not physically realistic, however, since for short T's other factors, such as O_2 diffusion time, are probably more important determinants of F_{ss} than calcium fluxes.

Fig. 6 shows the effects which changing certain parameters has on the steady-state interval-strength relation. The experiment described above—finding F as a function of T_i for atria contracting periodically at the steady state—was performed on guinea pig left atria; and the parameters a, b, f, and F_R were found. F_R and a were reasonably independent of T, while f and b were not (23). Furthermore, the calcium influx per beat, J_i, depends on T (16–18). The interval-strength curves in Fig. 6 for which f, b, and z are constant, serve only to demonstrate the qualitative behavior of the model.

DISCUSSION

We have developed a model for describing the interval-strength relationship in cardiac muscle and have specialized it to mammalian atrial muscle. The model has been shown to respond to several patterns of stimulation in a manner qualitatively similar to that of guinea pig atrial muscle. Although we do not claim that this agreement proves the assumptions underlying the model, we feel that it justifies further experimental testing of the model.

One of the attractive features of our model is its simplicity. The model requires few assumptions, and many of them are widely accepted. For example, Wood et al.

(11) have used several of the arguments presented here to explain the inotropic effects of electric currents on heart muscle. They have postulated the proportionality between $\{Ca^{+2}\}_i$ and F, and the control of $\{Ca^{+2}\}_i$ after activation by an intracellular calcium storage compartment which we have called n_1 (p. 436 of reference 11). They have also assumed that a fixed fraction, which we call f, of bound calcium is released at the onset of an action potential (p. 442 of reference 11). The principal new hypothesis presented here is the existence of n_2, a second calcium compartment which sequesters calcium after a contraction. The decay constant for the calcium level of n_2, b, is about 0.5 sec^{-1} (23). b is found by measuring changes in contractility (23). At the present time, no independent verification of the existence of n_2 or the value of b is possible. The parameters b, f, and z depend on the stimulus period T (16–18, 23). Since these parameters were held constant for the contraction staircases in Figs. 3, 4 B, and 5 B, a precise fit between the experimental and predicted results should not be expected.

Our model may have other limitations. (*a*) We have omitted feedback from our model, even though it probably exists (14, 23). For example, the variations of b, f, and z with T may result from feedback control. (*b*) We have considered the interaction of only two calcium storage compartments, when, in fact, tracer studies reveal several compartments (13–15, 18). Other compartments may be important under certain conditions, particularly during long rests. (*c*) We have considered the kinetics of only calcium. Although other ions—particularly sodium—surely affect the kinetics (14, 15), their influence on the kinetics should be fairly constant if their concentrations in the bathing medium are kept constant.

In addition to predicting some of the phenomena in the interval-strength relationship in mammalian atrial muscle, our model permits steady-state calculations of $n_1(\infty) = j_r/a$ which can be compared with experimental values. The total calcium content in guinea pig left atrium has been found to be about 2×10^{-6} moles/g. Although the percentage of this calcium available for the contractile process is not known, 25%, the percentage in the phase 2 compartment of dog papillary muscle (15) seems to be a reasonable estimate. Thus the calcium available to the contractile mechanism is about 5×10^{-7} moles/g. The calcium in n_1 is about j_r/a; a is about 5×10^{-3} sec^{-1} (23) for guinea pig left atrium. The j_r values for the same tissue measured by Winegrad and Shanes (18) and Grossman and Furchgott (16, 17) are 2.9×10^{-11} moles/g·sec and 1.7×10^{-9} moles/g·sec. The difference between these values—two orders of magnitude—is due to the differences between the techniques of the two authors. Grossman and Furchgott measured the ^{45}Ca uptake of the entire tissue, while Winegrad and Shanes washed out the tissue, taking the level of one of the slow ($T_{\frac{1}{2}} = 86$ and 168 min) calcium exchange phases to be the contractile calcium. Recent work by Little and Sleator (24) with guinea pig left atrium and by Langer (15) with dog papillary indicate that the component used by Winegrad and Shanes is not the one associated with the contractile process. It is therefore not surprising that j_r/a value based on j_r data of Grossman and Furchgott, 3.4×10^{-7}

moles/g, shows better agreement with the above estimate for the amount of contractile calcium than j_r/a values based on j_r data of Winegrad and Shanes, 6×10^{-9} moles/g.

The model presented here provides a physical meaning for the rested-state contraction, F_R. Equation 17 states that $F_R = Qfj_r/a$. This quantity is an important part of several of the theories discussed in the introduction.

Equation 17 can be solved for Q:

$$Q = \frac{F_R a}{f j_r}. \tag{42}$$

Equation 42 is rather remarkable. It gives the proportionality constant postulated above in terms of measurable quantities. Although F_R, j_r, and a depend on experimental conditions (23), an estimate of Q is possible. Guinea pig atria dissected, mounted, and superfused in the manner described by Hollander (25), have F_R values of about 1 g force or about 10^3 dynes per 50 mg tissue. $a = 5 \times 10^{-3}$ sec^{-1} (23), $f \cong 1$, and $j_r = 1.7 \times 10^{-9}$ moles/(g tissue)·sec (16, 17), Q is about 3×10^9 dynes (g tissue)/mole, or normalizing with respect to the average mass per tissue, 50 mg, $Q = 6 \times 10^{10}$ dynes/mole.

SYMBOLS

A, B	Coefficients in the equations predicting $n_1(t)$ and $n_2(t)$; moles/(g tissue).
a, b	Exchange constants in the equations predicting $n_1(t)$ and $n_2(t)$; sec^{-1}.
$\{Ca^{+2}\}_i$	Intracellular concentration of free calcium.
F	Maximum value of the developed tension; g force or dynes.
F_R	Rested-state contraction, or the contraction preceded by a very long interval.
f	Fraction of the calcium level n_1 released to the contractile mechanism.
j_{10}, j_{12}, j_{21}	Transmembrane and intracellular calcium fluxes; moles (g tissue)·sec.
j_r	Rest calcium flux into the cells; moles/(g tissue)·sec.
J_i	Amount of calcium entering the cells during an action potential; moles/(g tissue).
n_1, n_2	Two intracellular calcium storage compartments.
$n_1(t), n_2(t)$	Calcium levels of n_1 and n_2, respectively.
Q	Proportionality constant relating the amount of calcium contributing to a contraction to the force of the contraction.
T	Period of stimulation or interval between beats during periodic stimulation. T is also the period for paired-pulse stimulation.
T_p	Short interval during paired-pulse stimulation. The intervals between beats alternate between T_p and $T - T_p$.
T_i	Rest period after periodic stimulation.
w	fe^{-bT}.
x	Normalized value of F and n_1.
y	Normalized value of n_2.
z	Normalized value of J_i.

Dr. Manring's research was conducted in partial fulfillment of degree requirements while a doctoral candidate in the Department of Physics, Ohio State University.

Dr. Hollander was supported by United States Public Health Service Career Development Award HE 11,929.
This work was also supported in part by N.I.H. grant HE 09567, and the Central Ohio Heart Association.

Received for publication 23 September 1970 and in revised form 26 February 1971.

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