Mathematical Analysis of Oscillatory and Non-oscillatory Recovery of Contractility after a Rested-State Contraction and Its Modification by Calcium

By Christian J. Posner, Ph.D., M.D., and David A. Berman, Ph.D.

ABSTRACT

Recovery of contractility after a rested-state contraction in rat ventricle proceeded in two phases and is described by equations that are solutions of a nonhomogeneous second-order differential equation. The recovery can be characterized by a damping ratio defined in terms of the constants of the homogeneous portion of the differential equation. When calcium concentration was elevated, the usual smooth recovery curve underwent oscillatory variation and thus became less damped. There was a shift in phase between oscillation in recovery and in resting tension after a rested-state contraction. A control system is proposed to regulate relaxation and subsequent recovery of contractility. Possible mechanisms for an effect of calcium on such a control system are discussed.

ADDITIONAL KEY WORDS damping ratio resting tension nonhomogeneous second-order differential equation control system relaxation

Braveny et al. (1) demonstrated in guinea pig atria that restitution of contractility underwent oscillatory variation during conditions of low temperature. They also reported that high levels of calcium and epinephrine produced a similar alteration in the restitution process. We have independently studied this phenomenon in the rat ventricle strip and in the present communication show that the oscillatory variation in recovery of contractility is described by an equation that is a solution of a second-order differential equation. Previously, we presented an equation describing the recovery of contractility under conditions in which oscillatory variation does not occur (2). In the present study, the effects of alterations in the calcium concentration on the kinetics of the recovery of contractility after a rested-state contraction were investigated and the data are quantitatively expressed in terms of these equations.

Methods

Strips were cut from the outer wall of the right ventricle of male Sprague-Dawley rats (120 to 180 g) and suspended in medium of the following composition: 154 mM NaCl, 5.6 mM KCl, 1.1 mM CaCl₂, 5.5 mM glucose, and 1 mM sodium phosphate buffer; pH was adjusted to 7.4. The temperature was 27°C and the gas phase was 100% O₂. The tissue was attached to a Statham G10B-0.15-350 strain-gauge transducer and maintained at a resting tension of 0.75 g. The muscle was stimulated by a Grass model S-4 stimulator with monophasic pulses of 1.0 msec duration and above the threshold voltage. Isometric contractions were displayed on an Offner type R Dynograph recorder system equipped with type 9803 strain gauge couplers. During the initial equilibration period of 30 minutes the muscle was stimulated at 100/min.

The interval necessary to produce a rested-state contraction (3) was determined by successively prolonging the interval between beats until the force of contraction increased to a maximum and...
A. Time course of recovery of contractility after a rested-state contraction. The state of recovery is expressed as $F_t/F_{rc}$, where $F_t$ is the force of the test contraction and $F_{rc}$ is that of the rested-state contraction. The rested-state interval was 4 minutes, and the calcium concentration was 1.1 mM. The abscissas are the time intervals between the test and the rested-state contraction. The curve is a plot of equation 1 and is the mean of five experiments.

B. Graphical determination of the rate constants and coefficients of equation 1. (The data shown in A were replotted as log $(1 - F_t/F_{rc})$ against time.)

Results

Analysis of Recovery of Contractility after a Rested-State Contraction.—Rat ventricle strips were set up in 1.1 mM calcium medium and the recovery of contractility after a rested-state contraction was determined. The state of recovery of contractility at times less than the rested-state interval was taken to be the ratio of the force of the test contraction ($F_t$) to that of the rested-state contraction ($F_{rc}$) and was expressed as $F_t/F_{rc}$ (Fig. 1A). A plot of log $(1 - F_t/F_{rc})$ against time could be separated into two linear segments (Fig. 1B), which suggests that recovery of contractility proceeds in two phases and can be described by the following equation:

$$F_t = F_{rc} \left[1 - \left(C_1 e^{-k_1 t} + C_2 e^{-k_2 t}\right)\right],$$

where values of the rate constants ($k_n$) are calculated from the slopes of the two straight lines and the coefficients ($C_n$) from their zero time intercepts (2). The subscripts $n$ refer to the phase of the recovery period. The curve in
Figure 1A is a plot of equation 1 using the values of the rate constants and coefficients obtained by the method shown in Figure 1B. Equation 1 can be considered a solution of the following nonhomogeneous second-order linear differential equation with constant coefficients where $F = F_c/F_{cc}$.

$$\frac{d^2F}{dt^2} + a \frac{dF}{dt} + b F = A. \quad (2)$$

Such a solution is composed of two parts, a transient solution defined by the constants of the homogeneous portion of the differential equation ($a$ and $b$) and a steady-state solution defined by the forcing function ($A$). The transient solution consists of the exponential terms (in equation 1) and the steady-state solution is 1.0.

When the calcium concentration in the medium was increased from 1.1 mM to 6.6 mM, there was a period in the recovery of contractility when the test contraction amplitude underwent variation, initially increasing above, then decreasing below and finally returning to the rested-state value (Fig. 2A). Such a result would occur if the second-order system that describes the recovery kinetics became less damped allowing oscillatory behavior. The transient solution of equation 2 under conditions of oscillation is of the form:

$$e^{-kt} (D_1 \sin \omega t + D_2 \cos \omega t), \quad (3)$$

where $k$ is the constant determining the decaying exponential envelope of the damped oscillation which is of angular frequency $\omega$.
The data in Figure 2A were fitted by the following expression:

\[ F_t = F_{rc} \left[ 1 - (C_1 e^{-k_1 t} - C_2 e^{-k_2 t} \sin \omega t) \right], \]  

where the transient solution is \( C_2 e^{-k_2 t} \sin \omega t \) (the coefficient of the cosine term in equation 3 has been set equal to zero) and the steady-state solution is \( 1 - C_1 e^{-k_1 t} \). The value of \( \omega \) is equal to \( 2\pi / T \), where \( T \) is the period of oscillation of recovery taken from zero time. Rearrangement of equation 4 indicates that the values of \( k_2 \) and \( C_2 \) can be determined from the slope and intercept of a plot of \( \log \left( (F_t/F_{rc} - 1) (\sin \omega t)^{-1} \right) \) since at later times the influence of \( C_1 e^{-k_1 t} \) is negligible. This procedure is illustrated in Figure 2B. When the linear portion was extrapolated to zero time and the difference between this and the original data points was multiplied by \( \sin \omega t \) and replotted, another straight line was obtained. The values of \( k_1 \) and \( C_1 \) are obtained in the usual manner from this straight line. The curve in Figure 2A is a plot of equation 4 using data obtained by the method illustrated in Figure 2B. Thus equation 4 can be considered a solution of the above differential equation (equation 2) where \( Be^{-k_1 t} \) has been added to the forcing function. An alternative way of viewing the situation is to consider that the differential equation has two terms in the forcing function \( (A + Be^{-k_1 t}) \) and that under conditions without oscillation the value of \( B \) is zero. The behavior of the second-order system described by the differential equation can be quantitatively characterized in terms of a damping ratio \( (\xi) \) defined by the constants of the homogeneous portion of equation 2 \((\xi = a/2\sqrt{b})\). When \( \xi > 1.0 \), the system is heavily damped, aperiodic, and \( \xi \) equals \((k_1 + k_2)/2\sqrt{k_1 k_2}\); when \( 0 < \xi < 1.0 \), the system is lightly damped, oscillatory, and \( \xi \) equals \( k_2/\sqrt{k_2^2 + \omega^2} \). The transient solution thus determines whether the system is oscillatory or non-oscillatory.

**Effect of Calcium Concentration on Recovery of Contractility.**—Figure 3 shows the effect of calcium concentration on the time...
TABLE 1

Effect of Calcium on Kinetic Parameters of the Recovery of Contractility after a Rested-State Contraction

<table>
<thead>
<tr>
<th>Expts</th>
<th>Calcium (mM)</th>
<th>FRC (gm)</th>
<th>C1</th>
<th>C2</th>
<th>k1 (sec⁻¹)</th>
<th>k2 (sec⁻¹)</th>
<th>ω (sec⁻¹)</th>
<th>T (sec)</th>
<th>δ</th>
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<tr>
<td>A</td>
<td>1.1</td>
<td>0.96</td>
<td>0.49</td>
<td>0.33</td>
<td>0.60</td>
<td>0.05</td>
<td>1.88</td>
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<tr>
<td></td>
<td>2.2</td>
<td>1.24</td>
<td>0.55</td>
<td>0.24</td>
<td>0.79</td>
<td>0.07</td>
<td>1.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>1.49</td>
<td>0.62</td>
<td>0.17</td>
<td>0.82</td>
<td>0.11</td>
<td>1.55</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>4.4</td>
<td>1.62</td>
<td>0.68</td>
<td>0.11</td>
<td>0.94</td>
<td>0.12</td>
<td>1.58</td>
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<td></td>
<td>6.6</td>
<td>1.65</td>
<td>0.99</td>
<td>0.09</td>
<td>1.48</td>
<td>0.61</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>1.38</td>
<td>1.50</td>
<td>0.15</td>
<td>1.83</td>
<td>0.37</td>
<td>0.78</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.1</td>
<td>0.99</td>
<td>0.49</td>
<td>0.25</td>
<td>0.75</td>
<td>0.06</td>
<td>1.91</td>
<td></td>
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<tr>
<td></td>
<td>4.4</td>
<td>1.42</td>
<td>0.53</td>
<td>0.19</td>
<td>0.78</td>
<td>0.12</td>
<td>0.52</td>
<td>12.0</td>
<td></td>
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<tr>
<td></td>
<td>7.7</td>
<td>1.23</td>
<td>0.57</td>
<td>0.32</td>
<td>1.32</td>
<td>0.25</td>
<td>0.81</td>
<td>7.8</td>
<td></td>
</tr>
</tbody>
</table>

A is the mean of 5 experiments in which the calcium concentration was increased in small increments (Fig. 3); B is the mean of 4 experiments in which the calcium was increased in larger increments (Fig. 4). FRC is the value of rested-state contraction tension during period of determination of recovery of contractility. The coefficients (C1, C2) and the rate constants (k1, k2) were calculated by the methods described in Figures 1 and 2; ω is the frequency of the oscillation; T is the period of the oscillation.

A course of recovery of contractility after a rested-state contraction. It is evident that the recovery was accelerated when the calcium concentration was increased. The time course of recovery was a smooth curve at the lower calcium concentrations. However, when the calcium concentration was increased to 9.9 mM, an oscillation in recovery occurred. The kinetics of recovery of contractile tension were analyzed according to equations 1 and 4 and the results are given in Table 1. The rested-state contraction tension remained stable throughout the period of measurement of the recovery process. Increasing the calcium concentration over the range of 1.1 mM to 6.6 mM increased the values of k1 and k2. Thus calcium has an influence on the rate constants associated with both phases of the recovery period. Over the range of 1.1 mM to 6.6 mM calcium, increasing the calcium concentration increased the value of C1 and decreased C2. The rate constant and coefficient for the first phase of recovery in 9.9 mM calcium were greater than the corresponding values in 6.6 mM. However, due to the time required for the settling of the oscillation, the total recovery time was greater in 9.9 mM calcium than in 6.6 mM (Fig. 3). It is for this reason that k2 in 9.9 mM calcium was less than in 6.6 mM. It was observed that the magnitude of the calcium increment influenced the recovery kinetics. When the calcium concentration was increased in a larger increment, oscillations in the recovery period occurred at lower calcium concentrations (compare Fig. 3 with Figs. 2A and 4). When the calcium concentration was increased in a single increment from 1.1 mM to 4.4 mM, oscillation occurred (Fig. 4), and as shown in Table 1 the values of both rate constants were increased. Increasing the calcium concentration from 4.4 mM to 7.7 mM shortened the period of the oscillation and further increased the values of the rate constants and coefficients. Oscillations in recovery did not continue beyond a single period. Increasing the calcium concentration above that required to produce the oscillation (4.4 mM to 7.7 mM, Fig. 4), resulted in an increase in the value of C2 (Table 1). The effect of calcium on the kinetics of recovery of contractility can be described as a decrease in the damping of the system. In the case where the calcium concentration was increased in small increments, recovery passed progressively from the heavily damped state described by equation 1 to the lightly damped state described by equation 4. When the calcium concentration was increased in larger incre-
Effect of calcium concentration on recovery of contractility after a rested-state contraction. The calcium concentration was increased in relatively large increments in the same tissue. Each curve was fitted to the data using equation 1 or 4 and is the mean of four experiments. The abscissas are the time intervals between the test and rested-state contraction. Test contractions were placed randomly at intervals varying between 0.63 and 10 seconds after the rested-state contraction. The rested-state interval was 4 minutes in the experiments with 1.1 mm calcium and 2 minutes for the other calcium concentrations. The calcium concentrations were: solid circles = 1.1 mm; X = 4.4 mm; solid triangles = 7.7 mm.

Hyper-relaxation of a rested-state contraction in a ventricle strip suspended in medium containing a high calcium concentration (6.6 mm). Numbers refer to test interval (seconds). This trace was taken from the experiments shown in Figure 2A.

ments, the transition from the heavily damped to the lightly damped state occurred at a lower calcium concentration.

Relationship of Time Course of Recovery of Contractility to Variation in Resting Tension after a Rested-State Contraction.—In the presence of high calcium the resting tension after the rested-state contraction underwent a
CALCIUM AND CONTRACTILE RECOVERY

Figure 6

Change in tension during stress relaxation recovery (solid circles = developed tension; X = resting tension.) The rat ventricle strip was stimulated at a constant rate of 6/min. Prior to stress relaxation recovery, the muscle length had been increased to that producing maximum developed tension.

Change in Developed Tension during Stress Relaxation Recovery.—An attempt was made to simulate the length-tension alterations occurring during relaxation and subsequent oscillatory mechanical activity. For this study, rat ventricle strips were stimulated at a constant rate of 6/min. The muscles were stretched in increments after each sixth contraction until a length was reached at which maximum developed tension was attained. The muscle length was then rapidly decreased, and the time course of the secondary slow increase in resting tension (stress relaxation recovery) and the developed tension was observed. Figure 6 shows the time course of increase in developed tension during stress relaxation recovery. It is evident that the first contraction after the decrease in resting length (zero time, Fig. 6) had the lowest amplitude. The magnitudes of the contractions rapidly increased as the resting tension approached the plateau level. Thus it can be seen that conditions exist in which developed tension increases along with

period of variation, first decreasing below and later increasing above the precontraction value. The initial decrease in tension has been referred to as hyper-relaxation (4) and the secondary elevation in tension above the initial resting tension as after-contraction (5), or positive oscillation (4). When high speed traces were made with a high sensitivity of the recorder system during the determination of the kinetics of recovery of contractility, it was found that the transient overshoot occurred during the period of hyper-relaxation at a time when the tension was returning toward the resting level. The curve shown in Figure 5 was taken from the experiment whose recovery curve (6.6 mM calcium) is given in Figure 2A. It can be seen that the maximum overshoot occurred after a test contraction interval of 2.0 seconds, at a time when the tension was returning toward its resting value.

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a spontaneous increase in resting tension. This may be analogous with the increase in test contraction developed tension during the increase in resting tension after hyper-relaxation.

Discussion

Braveny et al. (1), in experiments with guinea pig atria, reported that the time course of the oscillations in restitution could be exactly correlated with the alterations in resting tension (after-contractions). They stated that the peaks in contractile tension during restitution occurred at times when the resting tension was at its minimum and concluded that the alterations in contractility were similar to those predicted by Starling's law. In the present study, we demonstrated in the rat ventricle strip that the peak of the overshoot did not occur when the resting tension was minimum, but rather when it was returning toward the original precontraction value. For this reason, oscillations in contractile activity during recovery under conditions that produce hyper-relaxation cannot simply be explained by the classical length-tension relationship implicit in Starling's law, but may implicate changes in the resting length-tension relation.

In view of the profound influence of calcium on the development of oscillations in resting tension and in the recovery, it seems reasonable to propose a mechanism whereby those processes which regulate relaxation mechanics also influence the time course of recovery of contractility after a contraction. Under normal circumstances the tension returns smoothly to its initial value after a contraction and the subsequent recovery is heavily damped. In the presence of high calcium the return of tension after a contraction undergoes damped oscillations and is followed by recovery which is also oscillatory. It is proposed that a dynamic biological control system regulates relaxation by exerting feedback constraints on the development of tension and thus ensures a smooth transition from activity to rest. Katzung (4) considered diastolic oscillations as the "hunting" of a feedback device for the previous resting length. According to our proposal each contraction would act as though it were a step input to the second-order system, which we have found describes the recovery kinetics. Alterations in the characteristics of the input (contraction) would manifest themselves as changes in the kinetics of recovery. Elsewhere (6, 7) we have quantitatively analyzed the time course of individual isometric contractions and the changes occurring in them during non-oscillatory recovery of contractility. Calculation of the damping ratio for test contractions indicated that its value was greatest early in the recovery period and that it decreased to the value of the rested-state contraction at the completion of the first phase of the recovery.

The mechanism by which calcium could exert its effect on such a control system is not known at the present time. One possibility is an action on the muscle cell membrane. Jensen and Katzung (8) have demonstrated a direct relation between simultaneously recorded oscillatory waves in membrane potential and resting tension after an action potential and stimulated contraction, respectively, in guinea pig atria beating in 10 mM calcium medium. Similar oscillations in electrical and mechanical activity were observed earlier by Bozler (9) and later by Kaufmann et al. (10). It is not clear, however, the manner in which the contractile apparatus would be stimulated by a change in membrane potential of the order of less than 10 mv (see Jensen and Katzung, Fig. 1) unless, in the presence of high calcium, the actomyosin is more sensitive to activation in the period immediately following the normal action potential. On the other hand, Reiter (5) failed to detect these electrical oscillations in the course of his investigation of after-contractions. Therefore one must consider a nonmembrane origin of this phenomenon. The oscillations in recovery of contractility appear to be associated with experimental interventions (calcium, epinephrine, low temperature) that increase calcium movement in the actomyosin space during the contraction.
Furchgott demonstrated that increased extracellular calcium (11) and norepinephrine (12) increase calcium exchange in contracting guinea pig atria. Kaufmann and Fleckenstein (13) suggested that a greater calcium influx followed prolongation of the action potential at low temperatures. It is of further interest that Goodall (14) was able to produce spontaneous oscillations in glycerinated muscle fibers in the presence of ATP-creatine phosphate solutions. A similar finding was that of Lorand and Moos (15) when phospho-enolpyruvate was the phosphate donor. The existence of oscillations in a system without a cell membrane is suggestive evidence for a direct effect of calcium on the contractile proteins, and this may be the site where the control system is operative. Perhaps further investigations in this area will help to elucidate the complex control mechanisms that direct the restoration of contractility after a given contraction and the return of tension following the onset of contractile activity.

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