Dynamic Stiffness of Cat Heart Muscle in Ba^{2+}-Induced Contracture

YASUTAKE SAEKI, KIICHI SAGAWA, AND HIROYUKI SUGA

SUMMARY We analyzed mechanical properties of kitten papillary muscles both at rest and in Ba^{2+} contracture by the frequency response method. The muscle length was perturbed sinusoidally, with an amplitude less than 0.3% of L_{max} over a frequency range from 0.1 to 60 Hz to determine the dynamic stiffness, F(\omega)/L(\omega), in which F(\omega) = amplitude of the force response wave, L(\omega) = amplitude of sinusoidal length wave, and \omega = frequency, and the phase shift of F(\omega) relative to L(\omega). In resting muscles, the dynamic stiffness increased minutely with increasing frequency and the phase relation showed a small lead over the entire frequency range. In muscles in contracture at low temperature (22-24°C), the stiffness first decreased with increasing frequency from about 0.2 to 1 Hz, then increased with a slope of 10-fold/decade, and finally plateaued over the range above 8 Hz. The phase relation showed a small lag between 0.3 and 0.5 Hz, but a clear lead of up to 60° between 0.8 and 16 Hz. With an increase in temperature to 36°C, the peculiar decrease in stiffness and the phase lag in the low frequency region decreased in size and shifted to a higher frequency region (about 4 Hz). These findings led us to two alternative, approximate analogues, which are similar to but simpler than that previously proposed for a twitching papillary muscle.

THE FREQUENCY method\textsuperscript{1} is used as a unique means to identify the dynamic properties of any given system. It has been applied to relaxed and contracting muscle\textsuperscript{2-7} by changing the length of muscle sinusoidally with a fixed small amplitude at various frequencies and plotting the amplitude ratio and phase relation of the force response waves to the length change waves as a function of frequency (Bode plot). From the features of the frequency-dependent amplitude ratio curve and phase curve, one can determine an abstract expression of the (mechanical) property of the muscle which is called the transfer function. One can further express the indicated frequency characteristics in terms of a mechanical analogue comprising such elements as spring, mass, and dashpot. However, for the frequency response method to be valid, the system to be studied must be linear and in a steady state. The linearity requirement can be met by using an extremely small amplitude of sinusoidal length change around a chosen mean length of muscle as the input (piecewise linearization). The steady state requirement can be met in skeletal muscle by studying tetanic contraction. In the case of heart muscle, special additional effort is needed because normally it twitches rhythmically and varies its characteristics in terms of a mechanical analogue similar to but simpler than that proposed for a twitching papillary muscle. Loeffler and Sagawa\textsuperscript{7} attempted to circumvent the difficulty associated with applying the frequency response method to normally twitching papillary muscle by using an envelope technique. Although this technique allowed them to estimate a spectrum of frequency responses between 0.1 and 35 Hz, the technique is fundamentally a compromise, and the relevance of the data can be questioned.\textsuperscript{9} Very recently, Steiger and his associates\textsuperscript{10} reported an extremely stable contracture of excised papillary muscle in Ba^{2+}-Tyrode’s solution. Such a steady contracture is an ideal condition for applying the frequency response analysis. Therefore, we used this contracture and repeated the frequency response analysis of papillary muscle properties to see if we could derive a mechanical analogue similar to that proposed for twitching heart muscle.

Methods

Preparation

Right ventricular papillary muscles were obtained from kittens (0.7-1.7 kg) anesthetized with chloroform. Immediately after the chest cavity was opened, the beating heart was quickly removed and placed in a dissection bath containing Tyrode’s solution (NaCl, 130 mM; CaCl\textsubscript{2}, 2.5 mM; KCl, 4.0 mM; MgCl\textsubscript{2}, 1.0 mM; NaH\textsubscript{2}PO\textsubscript{4}, 0.435 mM; NaHCO\textsubscript{3}, 10.0 mM, and dextrose, 5.56 mM) at room temperature and with a pH of 7.4. The right ventricular wall was cut open to expose papillary muscles and select a long and thin muscle appropriate for the study. The muscle length averaged 6.8 mm (from 5.5 to 9.5 mm) and the weight averaged 3.2 mg (from 2.0 to 4.5 mg). The muscle was tied at the tendinous and ventricular wall ends with 6-0 surgical silk thread, and excised from the ventricle.

The nontendinous end of the preparation was penetrated at the central side of the tie by a hook made of stainless steel wire (0.25 mm). The wire was then connected to a force transducer (Konigsberg model FS series).
which has a compliance of 0.1 μm/g. The temperature-dependent drift of the zero signal of this transducer was 150 mg/°C, and the sensitivity shift with temperature was much smaller than the nominal value of 0.4% of reading/°C. The tendinous end of the preparation was tied directly to a rigid stainless steel wire (0.4 mm). The other end of this wire was connected to the spindle of a linear vibrator (Ling Dynamic System, model 200) via a rod of a linear displacement sensor (Trans-Tek, Inc., model 241-000). The procedure generally was completed within 10 minutes after excision of the heart. The preparation, thus mounted in the bath, was superfused with Tyrode’s solution gassed with a mixture of 98% O₂ and 2% CO₂ at the desired temperature. A suction tube placed at a level in the bath maintained the fresh Tyrode’s solution flowing from the reservoir at that level. The muscle was stimulated with platinum plate electrodes by using electrical pulses of 3-msec duration and a voltage between 10% and 20% above the threshold.

All experiments were performed after a stabilization period of more than 3 hours during which the muscle was loaded with a resting tension of about 0.5 g and stimulated at a rate of 12/min under an isometric condition.

**Apparatus**

The apparatus used in this study is essentially the same as that reported by Pinto et al.11 Muscle length was monitored by the displacement sensor and controlled by the vibrator and a driver amplifier (Crown DC-300A) with the aid of a servo control circuit.11 The vibrator had a compliance of 0.05 μm/g when operated in the servo system. The overall compliance of the system (exclusion of muscle) was less than 1 μm/g. The frequency response of the length control system was flat to 100 Hz for a peak-to-peak amplitude of 100 μm, whereas the amplitude used in the experiment was less than 20 μm.

**Data Analysis**

A muscle was perturbed with sinusoidal length changes of a constant amplitude which was less than 0.3% of Lₘₐₓ. The frequency of sinusoidal length change ranged from 0.1 to 32 Hz. Either resting muscle or muscle in contracture responded with sinusoidal force waves to the sinusoidal length changes. We define the stiffness as F(ω)/L(ω), the ratio of amplitude of force response waves, F(ω), to the amplitude of the length changes waves, L(ω). We normalized the stiffness data for the same standard muscle (10 mm long and 1 mm² in cross-sectional area at Lₘₐₓ) that Loeffler and Sagawa7 used. The cross-sectional area of the muscle in contracture during lengthening was greater than the peak total twitch force during lengthening, whereas the opposite was true of the data during shortening. Likewise, the filled circles represent the total force of the muscle in contracture during lengthening (solid curve), whereas the solid triangles connected by a solid curve represent the data on peak, total twitch force (top) and contracture force (middle) of a muscle in the normal Tyrode’s and Ba²⁺ solution. In either solution the muscle was lengthened and shortened in a triangular fashion over a period of 15 minutes (bottom). We arbitrarily decided to determine Lₘₐₓ from the twitch force data during the lengthening. Figure 3 is a plot of the force-length relationship determined from the record shown in Figure 2. The solid triangles connected by a solid curve represent the data on peak, total twitch force during lengthening, whereas the solid triangles connected by a dashed curve show the force during shortening. Likewise, the filled circles represent the total force of the muscle in contracture during lengthening (solid curve) and shortening (dashed curve). In this example, the contracture force (which means the total force of the muscle in contracture) during lengthening was greater than the peak total twitch force during lengthening, whereas the opposite was true of the data during shortening. Thus, there was a far greater hysteresis loop in contracture than during twitch contraction. This was the case for all muscles studied. In the present study, we did not attempt to pursue the mechanism behind this interest-

![Image](https://example.com/image.png)
ing difference in the magnitude of hysteresis loop. The mean total muscle force of contracture generated by eight muscles in a 1 mM concentration of Ba$^{2+}$ was not statistically different from the mean peak total force during the twitch over the length range from $L_{\text{max}}$ to 0.88 $L_{\text{max}}$.

**Frequency Response Analysis**

The analysis was first applied to muscle resting in the normal Tyrode's solution (Fig. 4). The amplitude of the sinusoidal length perturbation, $L(\omega)$, was 18 $\mu$m (0.2% of $L_{\text{max}}$) peak to peak for this muscle (bottom panel of Fig. 4). The frequency was increased from 0.1 to 0.2, 0.4, 0.8, 1, 2, 4, 8, 16, and 32 Hz. The middle panel shows the total muscle force responding to the length perturbations, whereas the top panel indicates the sinusoidal force response after a 10-fold amplification. The amplitude of the force response, $F(\omega)$, of muscle resting in the normal Tyrode's solution changed only very slightly with the increase in frequency of perturbation. In contrast, when the same muscle was in the state of contracture (Fig. 5), the amplitude of the force response first slightly decreased with an increase in frequency up to 1 Hz but then markedly increased with further increase in frequency. To present the frequency dependence of the amplitude ratio, $F(\omega)/L(\omega)$, or dynamic stiffness, and the phase shift of the force wave with respect to the length wave, we plotted the two variables in the form of Bode plot.¹

Figure 6 shows a representative Bode plot of the dynamic stiffness (top) and the phase shift (bottom) obtained from one muscle. The filled circles represent the data from the muscle resting in the normal Tyrode's solution. The increase in stiffness of this resting muscle was only 2.7 g/mm (from 8.8 to 11.5 g/mm) with an increase in frequency from 0.1 to 32 Hz. The phase relation showed a barely measureable degree of lead over the entire frequency range.

When the muscle was in Ba$^{2+}$ contracture (open circles in Fig. 6), the stiffness first decreased from 15 to 9 g/mm with an increase in frequency from 0.2 to 0.8 Hz, but then increased and finally plateaued at a level of about 50 g/mm over the frequency range above 8 Hz. The phase relation showed a mild lag of the force response between about 0.3 and 0.5 Hz, but a clear lead over the range between 0.8 and 16 Hz. The maximum lag was about $-10^\circ$ at 0.4 Hz, whereas the maximum lead was about $60^\circ$
at 2 Hz for this muscle. These frequency characteristics of dynamic stiffness and phase shift were common to all eight muscles studied.

The phase lag in the low frequency region also was recorded on the oscilloscope in terms of Lissajous' figure. The ordinate of the tracings in Figure 7 represents the force and the abscissa the length of the same muscle as that shown in Figure 6. At the low frequencies of 0.1 and 0.2 Hz, there was neither lead nor lag; the Lissajous' image was a straight line. At 0.4 and 0.5 Hz, the force response lagged behind the length change; thus the beam drew a loop rotating counterclockwise. This loop disappeared at 0.6 Hz, a straight line appearing again. At the higher frequencies, the force response led the length change, resulting in a clockwise rotating Lissajous' loop.

Figure 8 plots the mean values and the standard errors of dynamic stiffness determined at 13 frequencies for eight muscles in contracture at $L_{\text{max}}$. The resting dynamic stiffness of these preparations at $L_{\text{max}}$ was 12.1 ± 2.7 g/mm (mean ± standard error) at 0.1 Hz and 15.0 ± 2.8 g/mm at 32 Hz. Because of this relatively small frequency dependence of resting muscle stiffness, the features shown in the plot can be considered to derive mainly from the active muscle elements. The features are that (1) the stiffness is relatively independent of frequency in the high and low frequency regions, and (2) the stiffness decreases and then increases with an increase in frequency in the intermediate range of frequency. The dotted line indicates a theoretical stiffness curve expected for a first order system with break frequencies at about 1.5 and 7 Hz. The similarity of the mean data to the dotted line is obvious except in the frequency region between 0.4 and 1.5 Hz in which a mild decrease in stiffness and a small phase lag occurred.

Stiffness-Frequency Relation at Different Muscle Lengths

Figure 9 shows a set of stiffness-frequency relation curves obtained from a single muscle at two different
lengths, 0.98 and 0.96 L_{\text{max}}. It can be noted from these curves that the absolute value of the dynamic stiffness is dependent on muscle length. However, the shape of these curves is almost independent of muscle length and shows only parallel vertical shifts with the change in muscle length. This also was true of the stiffness-frequency relation for resting muscle; there was little difference in the shape of the relation with changes in muscle length. The absence of the effect of length on the shape of stiffness curve was confirmed in three other muscles at lengths between 0.96 L_{\text{max}} and L_{\text{max}}. Below 0.95 L_{\text{max}} the force response signal to L(\omega) became so small at low frequencies that the curve shape could not be determined accurately.

**Effects of Temperature on the Stiffness-Frequency Relationship**

Figure 10 shows a typical example of the effects of changes in temperature on the stiffness-frequency relationship of a muscle in Ba^{2+} contracture. At 22°C, the peculiar dip in stiffness occurred in a frequency region between 0.6 and 0.8 Hz. The decrease in stiffness was quite marked. With an increase in bath temperature to 36°C, the dip of the stiffness curve shifted to a higher frequency region from about 4 to 8 Hz, while the depth greatly decreased. The increase in temperature also caused a drastic reduction in the dynamic stiffness at both the low and high frequencies. However, the magnitude of decrease in stiffness was different at the two frequency limits. At 40 Hz, the decrease was about 60 g/mm, a reduction to one-quarter of the stiffness at 22°C. At 0.1 Hz, the decrease in stiffness amounted only to about 10 g/mm, a reduction of one-half of the value at the low temperature. Whether the phase response showed a cor-
Stiffness-Force Relationship at Different Lengths

Figure 12 shows a plot of dynamic stiffness at 32 Hz as a function of mean muscle force in a resting muscle and the same muscle in Ba²⁺ contracture. There is a significant difference in the slope of the stiffness-muscle force relationship between the resting condition and the contracture state. The broken line was drawn through the three points that were obtained from the resting muscle at three different lengths (L_max, 0.98 L_max, and 0.96 L_max). The solid lines were drawn through three pairs of stiffness data in the resting and contracture state at the three lengths. The relation between resting stiffness and resting force is much steeper than between active stiffness and active force.

Discussion

Resting Muscle

The presence of a viscous property in resting (relaxed) heart muscle has been recognized by several investigators. Loeffler and Sagawa peeled two time constants (about 0.1 and 1.5 seconds) from the transient force response to a relatively large step change in length (1.2% of L_max) given during diastole of periodically twitching papillary muscle. Because of this finding, the passive branch of their model comprises three elastic elements and two viscous elements. In the present study, too, we could trace in relaxed papillary muscle a similar transient (viscoelastic) response to a small step change in length (0.2% L_max). However, the response magnitude was so much smaller than in the previous study that it was difficult to decompose the response into single, double, or multiple exponential curves with confidence. In the frequency response analysis, the dynamic stiffness of the resting muscle changed only very slightly within the investigated range of frequency and indicated no clear-cut break frequency.

The difference between results of the present and earlier studies could derive, at least in part, from the absence of the twitch in the present preparation and its presence in the earlier study. Several observations

![Figure 9](image_url)  
**Figure 9.** Effects of muscle length on the stiffness-frequency relationship. The solid lines connect total stiffness data obtained from a muscle in contracture. The dotted lines are for the data from the same muscle in resting state.

![Figure 10](image_url)  
**Figure 10.** Effects of temperature on the stiffness-frequency relationship in a muscle in contracture.

responding shift in the frequency at which the peculiar lag occurred was difficult to determine because of the small force signals at the high temperature. In resting muscle, the stiffness-frequency relationship curve shifted vertically downward by roughly 4 g/mm with increase in temperature (Fig. 11). Thus dynamic stiffness decreased with temperature in both resting and active muscle, but the decrease in the latter was much greater. We confirmed a similar effect of temperature in two other muscles.
The relations exhibited in Figure 12 indicate to us that earlier paper, we consider that the passive property of muscle does not transform itself into the active property when muscle is twitching. As discussed in detail in the previous study, of the dynamic stiffness of resting muscle at 32 Hz was plotted against the mean muscle force, the relation was linear and the slope of the relation line was different from (actually greater than) the similar relation line between the instantaneous dynamic stiffness of contracting muscle and its instantaneous force. The velocity of length change in the present study at 32 Hz with a length perturbation of 0.2% Lmax was 4 mm/sec which is still smaller than 2 muscle length/sec. This is likely to be the reason for the almost flat frequency characteristics of resting muscle. Thus, for the limited frequency range investigated, we assigned a single elastance for the passive branch of the model shown in Figure 13.

Relation between Resting and Active Muscle Stiffness

The basis for a parallel combination of the passive and active branches in Loeffler and Sagawa's model was that, when the dynamic stiffness of resting muscle at 32 Hz was plotted against the mean muscle force, the relation was linear and the slope of the relation line was different from (actually greater than) the similar relation line between the instantaneous dynamic stiffness of contracting muscle and its instantaneous force (see Fig. 11 of Reference 7). Exactly the same finding is indicated in Figure 12 of this paper. Of course the muscle in the present study was in a steady state of contracture, and for this reason there is only one data point obtained for active muscle at a given length instead of the multiple points which can be obtained when muscle is twitching. As discussed in detail in the earlier paper, we consider that the passive property of muscle does not transform itself into the active property but rather persists as it is throughout twitch or contracture. The relations exhibited in Figure 12 indicate to us that the dynamic stiffness of resting muscle establishes the starting point for the further increases in dynamic stiffness and in muscle force as muscle is activated. In terms of the model, when muscle is activated, the active branch joins in parallel with the passive branch instead of replacing the latter. Templeton et al. once reported that the slope for activated muscle was identical to that for resting muscle. However, in a more recent study, they recognized the difference in slope between the two relation lines as shown in Figure 12. Based on these findings, we maintain the notion of a parallel combination of the passive branch with the active branch in our model.

Muscle in Ba2+ Contracture

The main features of the dynamic stiffness-frequency relation curve stated in connection with Figures 6 and 8, namely, two plateaus at the low and high frequency regions and an increase of stiffness in the intermediate frequency region, are identical to the features recognized in twitching papillary muscle in the previous study (compare Figure 8 with Figure 8 of Reference 7). These features of the "gain" curve of the Bode plot are characteristic of a first order lead-lag system. However, there is another feature which requires us to refrain from a quick identification of the active muscle as a first order system. It is the peculiar dip in the dynamic stiffness curve over the frequency range from about 0.4 to 1.5 Hz and the phase lag in the similar range of frequencies. Perhaps a clear identification of this feature became possible in the present study because of the extremely stable activated state during Ba2+ contracture, but was impossible in the previous study in which muscle was twitching every 6 seconds and, therefore, the frequency response analysis in the low frequency range was quite difficult. If we disregard this feature, which became insignificant at body temperature anyway, we can propose two alternative analogues for the active branch of the model (Fig. 13). We emphasize that they are not unique but are the simplest among those which are consistent with the observed frequency characteristics.

From the equations described in the Appendix and the Bode plot in Figure 8, we evaluated the average values of the system parameters. In the estimation, we disregarded the dip in the gain curve of the Bode plot. The results are listed in Table 1 for two types of analogue. Judging from the developed force, the contracture with 1 mm concentra-
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Table 1  System Parameter Values for the Two Analogues in Figure 13

<table>
<thead>
<tr>
<th>Parameters</th>
<th>K0 (g/mm)</th>
<th>K0 or K* (g/mm)</th>
<th>K0 or K0' (g/mm)</th>
<th>C or C* (g sec/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model type 1</td>
<td>12.1</td>
<td>3.4</td>
<td>28.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Model type 2</td>
<td>12.1</td>
<td>3.8</td>
<td>31.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The data used for estimation of these values are the same as plotted in Figure 8, obtained at Lmax and 24°C.

Effects of muscle length on the model elements can be estimated from the set of frequency-stiffness relationship curves shown in Figure 9. First, K0, which is identified as \( \lim_{\omega \to 0} K_T(\omega) \) in Model type 1, was almost independent of the muscle length, since the difference between the dynamic stiffness at low frequency and at high frequency was virtually independent of the muscle length. Second, \( C(=KJ2\pi f) \) was affected little by muscle length since \( f_1 \) and \( K_0 \) were almost independent of the muscle length. Third, \( K \) (the difference in stiffness at 0.1 Hz between resting muscle and muscle in contracture) was also insensitive to change in muscle length. In contrast, \( K_0 \), the elastance of resting muscle, was highly sensitive to change in muscle length.

We also can evaluate the effect of temperature on the values of the model system parameters. With an increase in temperature, the dynamic stiffness value decreased over both the low and high frequency regions. Therefore, for model type 1, both \( K_0 + K \) and \( K_0 + K + K_0 \) must have decreased with temperature (see Equations 3 and 4 in Appendix). However, since the high frequency stiffness value decreased much more than the low frequency value, we must infer that \( K_n \) decreased far more than \( K_r + K \). There is another inference we can make in continued reference to Model type 1. Keeping in mind Equation 6 for the higher break frequency (\( f_2 = K\sqrt{2\pi f} \)), the fact that both break frequencies shifted to higher values indicates that C decreased even more greatly than \( K_n \) with the increase in temperature. This inference is supported by a recent study by Templeton et al.17 The inferences for Model type 1 also are true for Model type 2, because there are the same unique algebraic relations between the present two models, as discussed in Appendix B of our previous paper.7

Most intriguing is the peculiar dip in the stiffness-frequency relationship curve and the phase lag of the force response behind the length change in the frequency region between 0.4 and 1.5 Hz at 21-24°C. The same phenomenon was documented also as the counterclockwise rotation of the Lissajous' loop (Fig. 7). This means that the viscous modulus of muscle becomes negative at those frequencies and that one obtains a positive net energy from the muscle while he stretches and shortens it. Such an energy-producing cycle has been noted in insect flight muscles,16,19 in glycerinated skeletal muscles,20 and in glycerinated4 and living8 heart muscles of the rabbit and cat. The investigators suggested that both this response and the delayed force response to a quick step change in muscle length probably reflect the kinetics of the attachment and detachment of cross bridge in response to an increase and decrease of filament stress and perhaps also strain. Steiger3 reported that the response was very sensitive to temperature. In the present study, the frequency at which the bottom of the nadir of the stiffness curve occurred shifted from about 1 Hz to 4 Hz when the temperature was increased from 22°C to 36°C. This seems to be consistent with previous findings.21

Thorson and White2 and Abbott4 studied the mechanical properties of glycerinated insect flight muscle with special emphasis on the peculiar frequency characteristics over those frequencies in which force response lags behind length change. They attempted to explain it by a simple two-state model of the crossbridge kinetics which is very much similar to Huxley's 1957 model.22 Their equation (e.g. Equation 4 in Reference 4) represents the first order delay system which explains the reduction of dynamic stiffness and the lag of force response above a certain frequency. However, it does not explain why the delay decreases as the frequency further increases, instead of approaching -90° asymptotically, and why the stiffness increases to another plateau instead of attenuating toward an infinitesimally small value with frequency. Thorson and White2 appear to ascribe the characteristics in the high frequency region to the viscoelastic properties of the bound crossbridge, the myofilaments, and other connective tissues in the muscle. However, they have not identified the mechanical property of these elements as clearly as for the low frequency range characteristics.

Whatever the mechanism for the decrease in stiffness with phase lag in a certain frequency region, it does not seem to have an important physiological implication in the case of heart muscle, because it is greatly attenuated at normal body temperature (Fig. 10). On the other hand, it may be a potential clue to the understanding of the mechanism of muscle contraction, since the phenomenon is observed in a variety of types of muscles.20

In the proposed model, the frequency-dependent increase in dynamic stiffness over the intermediate range is ascribed to the viscous element C or C'. This finding for muscle in contracture presents an interesting contrast to the recent findings for muscles in the state of rigor.26,27 For example, Steiger and his associates18 found that, for cat papillary muscle in rigor, the dynamic stiffness increased only slightly with an increase in frequency from 0.05 to 30 Hz, and that the absolute value of the stiffness in rigor muscle was greater than that of muscle in contracture over the entire frequency range. One of the possible reasons for the presence of two plateaus in the Bode plot
of muscle in contracture but a single plateau for muscle in rigor may be the following. In rigor, the crossbridges responsible for the production of force are said to be fixed to the actin filaments in the "arrow-head" position. Heinl et al. showed that the crossbridges in the "arrow-head" position could not detach or attach, nor could they rotate on the actin filament. Muscle in such a condition (rigor) will not exhibit an apparent viscous behavior nor a frequency-dependent stiffness. By contrast, viscosity (C or C') responsible for the increase in stiffness of muscle in contracture derives probably from the finite rate of crossbridge turnover and the finite rate of rotation of the crossbridge heads.

APPENDIX

The equations below represent the behavior of a muscle in contracture. They appear simpler than those for our earlier model because of the simpler passive branch. However, in fact, they are by far simpler in that the system parameter values are time invariant as opposed to the time-varying elastic and viscous elements in the earlier model.

In Case of Model Type 1

The transfer function from the input length change \( \Delta L(s) \) to the force response \( \Delta F(s) \) is (S: Laplacian operator)

\[
\frac{\Delta F(s)}{\Delta L(s)} = (K_n + K) \left\{ \frac{1 + \frac{K_s + K_n + K}{K_s (K_n + K) S}}{1 + \frac{C}{K_s S}} \right\}
\]

The total dynamic stiffness (gain) is:

\[
K_T(\omega) = \lim_{s \to j\omega} \left| \frac{\Delta F(j\omega)}{\Delta L(j\omega)} \right| .
\]

The approximations for the low and high frequency regions are:

\[
\lim_{\omega \to 0} K_T(\omega) = K_n + K
\]

the dynamic stiffness at 0.1 Hz. and

\[
\lim_{\omega \to \infty} K_T(\omega) = K_n + K + K_s
\]

the dynamic stiffness at 32 Hz. The lower break frequency is given by

\[
f_1 = \frac{K_s (K_n + K)}{2\pi C (K_n + K + K_s)} \text{ (Hz)},
\]

and the upper break frequency is given by

\[
f_2 = \frac{K_s}{2\pi C} \text{ (Hz)}.\]

In Case of Model Type 2

The transfer function is:

\[
\frac{\Delta F(s)}{\Delta L(s)} = \frac{K_n (K_s + K) + K_s K_n S C}{K_s K' + K_s K_n S + K_s K_n S C} S
\]

The dynamic stiffness in the low frequency region is:

\[
\lim_{\omega \to 0} K_T(\omega) = \frac{K_s K'_n + K'_s K_n}{K'_s + K'_n} .
\]

whereas that in the high frequency region is

\[
\lim_{\omega \to \infty} K_T(\omega) = K_n + K'_s .
\]

The lower break frequency is given by

\[
f_1 = \frac{K_s K'_n + K'_s K_n}{2\pi C (K_n + K'_n)} \text{ (Hz)}.\]

The upper break frequency is given by

\[
f_2 = \frac{1}{2\pi C} \text{ (Hz)}.\]

Acknowledgments

We are deeply indebted to Drs. G.J. Steiger and A.J. Brady, University of California, Los Angeles, for providing us with detailed information on Ba*+ contracture in cat papillary muscle. We also acknowledge that Dr. Steiger was kind enough to make several of his manuscripts on similar studies available to us.

References

The Effects of Ouabain on the Transmembrane Potentials and Intracellular Potassium Activity of Canine Cardiac Purkinje Fibers

Dennis S. Miura and Michael R. Rosen

SUMMARY  Open-tip microelectrodes containing a potassium-sensitive liquid ion exchanger (Corning 477317) were used to study the effects of ouabain on the intracellular potassium activity and the transmembrane potentials of beating canine cardiac Purkinje fibers. The preparations were superfused with Tyrode’s solution containing ouabain, $2 \times 10^{-7}$ M, and potassium, 4 mM, for 30 minutes. At the end of this period, intracellular potassium activity had decreased from the control value of 130.0 mM to 112.2 mM. The resting membrane potential determined through conventional 3 M KCl-filled microelectrodes decreased from $-83.6$ to $-78.8$ mV. Comparison of the decrease in the potassium equilibrium potential with the decrease in the resting membrane potential suggests that there was an accumulation of potassium at the exterior surface of the cell membrane. The effect of ouabain on the resting membrane potential, therefore, was due to a change in the transmembrane potassium ion gradient. This, in turn, resulted from a decrease in intracellular potassium activity and, apparently, from an increased potassium activity at the cell surface.

STUDIES OF the effects of digitalis on the electrophysiological properties of mammalian cardiac fibers have revealed a close relationship between digitalis and potassium ion. In 1963, Kassebaum reported that G-strophanthin, $1.4 \times 10^{-6}$ M, had a biphasic effect on the current-voltage relationship of sheep Purkinje fibers: initially membrane permeability to potassium was increased; later, it was increased. This result was similar to that in an earlier report by Dudel and Trautwein. Kassebaum interpreted the decrease in potassium permeability as explaining the initial digitalis-induced prolongation of the action potential duration (APD) and the later increase in permeability as explaining the associated decrease in APD. The exposure to G-strophanthin that resulted in a decrease in APD also brought about a decrease in resting membrane potential.

Other investigators have used indirect methods to determine the effects of digitalis on the intracellular potassium concentration. On the basis of these studies, it appears that at low concentrations of digitalis— or following short periods of exposure to digitalis— there is no change (or a slight increase) in intracellular potassium concentration, but at higher concentrations there is a decrease in intracellular potassium concentration. The loss of intracellular potassium and accompanying decrease in membrane potential that occur at high or toxic concentrations of digitalis have been attributed to poisoning of Na+-K+-activated ATPase by digitalis.

Despite these studies of the effects of digitalis on membrane permeability, potassium concentration, and membrane potential, direct measurements of the changes in intracellular potassium activity ($a_k$) induced by clinically relevant toxic concentrations of digitalis have not
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Circ Res. 1978;42:324-333
doi: 10.1161/01.RES.42.3.324

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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