The Hyperthyroid Heart
An Analysis of Systolic and Diastolic Properties in Single Rat Ventricular Myocytes

Richard A. Josephson, Harold A. Spurgeon, and Edward G. Lakatta

Single ventricular myocytes were isolated by collagenase digestion from the hearts of 6–8-month-old male Wistar rats in either the control (euthyroid) state or after 7 days of daily injection of 0.64 mg/kg thyroxine (hyperthyroid). Myocytes were field-stimulated from slack length, and contraction was measured with an inverted microscope–photodiode array–computer apparatus. The effect of pacing rate and ouabain administration on systolic and diastolic function was examined. Single myocytes isolated from hyperthyroid hearts maintain the properties of bulk muscle, because maximal twitch velocity is augmented 98% and the time course of contraction as measured by the time to peak shortening, relaxation time, or contraction duration is abbreviated 39%. Spontaneous sarcoplasmic reticulum calcium release, as measured by the occurrence of contractile waves, is increased in the hyperthyroid myocytes. This increased frequency of spontaneous sarcoplasmic reticulum calcium release is most marked under conditions known to be associated with high intracellular calcium, such as low pacing rates or digitalis glycoside administration. It can account for the hypoperformance of the hyperthyroid myocytes noted under these conditions because it is associated with depletion of sarcoplasmic reticulum calcium stores and diminution of subsequent twitch amplitude. These observations may help explain, in part, the cellular basis of the altered cardiac performance in the hyperthyroid state. (Circulation Research 1990;66:773–781)

Previous studies have demonstrated that characteristic biochemical and functional alterations of the myocardium of many species are induced by the hyperthyroid state.1–8 The rat model has been fairly well characterized in this regard.9–12 Daily intramuscular injection of adult (500–600 g) rats with thyroxine (6.4 mg/kg initial body wt/day for 7 days), which produces a marked hyperthyroid state, increases serum thyroxine levels 10-fold, decreases body weight 10%, increases left ventricular weight 35%, and shifts the myosin isoenzyme distribution to predominately V1.9 Electrophysiological study of isolated muscle indicates that the hyperthyroid state as produced above prolongs the action potential duration by approximately 50%.11 The velocity of calcium accumulation of isolated sarcoplasmic reticulum (SR) vesicles increases 50%10 in this hyperthyroid state. These structural, electrophysiological, and biochemical alterations are associated with a modest, but variable, increase in developed tension, approximately a doubling of the rate of tension development, and 35% shortening of the time to peak tension, half-relaxation, and contraction duration.9

A decreased threshold for catecholamine stimulation10,11 has also been demonstrated. Under some conditions, the hyperthyroid heart appears to perform less well than its euthyroid counterpart.13–15 Paradoxically, the maximum contractile response to inotropic stimulation, such as by isoproterenol or glycosides, may be decreased in the hyperthyroid state.10,13 Diastolic properties also appear to be altered.16,17 The cellular mechanisms underlying these phenomena, which can adversely affect myocardial function, are incompletely understood.

Prior studies from this laboratory18–20 have shown that single left ventricular myocytes isolated from adult (euthyroid) rat heart mimic many of the contractile properties of bulk tissue and, in addition, provide unique insights into diastolic events that may be obscured in other preparations. In the present studies, we have isolated single left ventricular myocytes from hyperthyroid and euthyroid rats and measured systolic and diastolic properties.

Several lines of evidence show that intact rat cardiac muscle21,22 and single myocytes,18,19 when unstimulated and bathed in 1.0 mM calcium, apparently exhibit a high degree of SR calcium loading.
This is manifest as a rested-state twitch, which is the maximum obtainable, and the occurrence of spontaneous SR calcium release. This spontaneous calcium release occurs locally within a cell and causes localized activation and contraction of adjacent sarcomeres; it then spreads via calcium-induced calcium release and is manifest as a propagating contractile wave. There is evidence to suggest that spontaneous calcium release may exert profound effects on myocardial function. The resultant oscillations in myoplasmic calcium cause small depolarizations of the cell membrane, which under certain circumstances may cause spontaneous action potentials, and presumably dysrhythmias. Spontaneous SR calcium release may lead to an increase in diastolic tone and may diminish twitch amplitude. We hypothesized that the altered SR and cell calcium loading of hyperthyroid myocytes may profoundly affect their diastolic function by lowering the threshold for spontaneous calcium oscillations to occur.

Materials and Methods

Myocyte Isolation

Male Wistar rats (6–8 months old) were obtained from the aging colony of the Gerontology Research Center and treated with intramuscular thyroxine (6.4 mg/kg body wt/day for 7 days; Sigma Chemical, St. Louis, Missouri); they were killed on the eighth day by guillotine decapitation. The hearts were rapidly excised, the ascending aorta was cannulated, and the heart was retrogradely perfused at 37°C with solution A (see Table 1 for composition of solutions). When the perfusate became clear (after approximately 15 ml solution A), the heart was perfused with solution B. When the heart became soft (approximately 20–45 minutes), the perfusion was terminated, the left ventricle and septum were dissected free, minced, and filtered through 200-μm nylon gauze, and the cells were harvested by gravity sedimentation. The pellet was then resuspended in solution C (solution A with 250 μM \([\text{Ca}^{2+}]\)), and the cells were sedimented again. Cells were then resuspended in either solution D or solution E. Those in solution D were plated onto 35 mm Petri dishes that had been precoated with poly(2-hydroxyethyl methacrylate) (polyHEMA, Aldrich Chemical, Milwaukee, Wisconsin). Cells plated on the dishes precoated with solution D were lightly attached to the dish at their midportion; the region of attachment was approximately 10% of the total cell length. Cells plated in the dishes that were precoated with polyHEMA floated free in solution E.

Contractile Measurements

Petri dishes were placed on the stage of an inverted microscope (model IM-35, Carl Zeiss, Thornwood, New York) and superfused with solution E at 3 ml/min. To change superfusate (i.e., drug concentrations), a new stock solution of the desired composition was attached to the inlet of the superfusion pump, and a minimum of 10 dish volumes (i.e., ≥10 minutes) was superfused before obtaining measurements. The cells were field-stimulated with a Grass stimulator (Grass Instrument, Quincy, Massachusetts) via two platinum wire electrodes. The microscopic image light was split into three pathways. Both direct observation and recording of a television image were thus possible in two of these pathways. The third light path was directed onto the surface of a charge-coupled photodiode array (1024 elements, reticon 1024G/RC100B, EG&G Reticon, Sunnyvale, California), which was interrogated asynchronously at 5-msec intervals. With the magnifications typically employed in our microscope setup, each diode senses light from approximately 0.25 μm. A specially designed combination of analog and digital circuitry was employed to determine the changes in density corresponding to the beginning and the end of the cell, to count the number of diodes corresponding to the distance between beginning and end of the cell image, and to provide a signal proportional to cell length updated after each scan. Cell length signals thus obtained have a spatial resolution of 0.25 μm and a temporal resolution of 5 msec because, unlike video analysis, there is no appreciable memory component in the diode array. Cell length thus obtained was digitized and recorded (VAX-730, Digital Equipment, Marlboro, Massachusetts) on-line for subsequent analysis.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of Solutions</th>
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<tbody>
<tr>
<td>Solution</td>
<td>Earle's</td>
</tr>
<tr>
<td>A</td>
<td>+</td>
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<tr>
<td>B</td>
<td>+</td>
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<tr>
<td>C</td>
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Earle’s, Earle’s balanced salt solution (GIBCO Laboratories, Grand Island, New York) with 0.8 mM MgSO₄; added: DMEM, Dulbecco’s modified Eagle’s medium (GIBCO Laboratories) containing (mM) NaCl 109.6, NaHCO₃ 44.0, NaH₂PO₄ 0.9, KCl 5.4, MgSO₄ 0.7, glucose 25.0, and CaCl₂ 1.8; FCS, fetal calf serum (Hyclone Laboratories, Logan, Utah); HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (Sigma Chemical) containing (mM) NaCl 137, MgSO₄ 1.2, KCl 5.0, HEPES 20, and D-glucose 16, pH 7.4.
All data were computed on a beat-by-beat basis according to the following definitions: Resting cell length was the average of three samples immediately before each stimulation and was the only parameter averaged within each beat. Peak shortening was taken as the minimum cell length recorded in a given twitch, and time to peak shortening was the time from stimulation to peak shortening. Maximal shortening velocity was computed by best-fit successive approximation of the local slope of the length-versus-time signal using a short segment of the available data incremented in 5-msec steps from rest to peak. Relaxation time was taken as the time from peak shortening to 50% recovery to resting length. Contraction duration, following our previous convention,9,11 was expressed as the sum of time to peak shortening and relaxation time.

We have found in other experiments28 that the twitch parameters measured in unattached cells (in the polyHEMA-coated dishes) do not statistically differ from those measured in cells that were lightly attached (in the dishes precoated with buffer D). Therefore, we performed all reported measurements on lightly attached cells, because it was much easier to function over long (1–2-hour) periods of time when cells maintained a stable position in the field of view.

We first measured baseline contractile parameters of isolated euthyroid and hyperthyroid myocytes under the same conditions as we have previously employed for bulk muscle (29°C, stimulation at 24/min).11 After thus validating the model, we measured the frequency of spontaneous SR calcium release as manifest by the occurrence of spontaneous contractile waves. We then sought to determine the relation of diastolic SR calcium release to stimulated twitches under various states of intracellular calcium loading. These latter experiments were performed at physiological (37°C) temperature.

Basal twitch parameters were measured during 10 steady-state beats (see below) per myocyte, and the values thus determined were averaged to yield the mean values for that myocyte. The measurements were repeated in 15 myocytes or more per heart in each of five hearts of rats of each type (euthyroid and hyperthyroid). Intraheart values and values among rats were analyzed by an analysis of variance. Intraheat values were averaged after such analysis and did not demonstrate any significant (p<0.05) intraheart differences.

### Results

**Table 2. Twitch Parameters**

<table>
<thead>
<tr>
<th>State</th>
<th>Resting cell length (μm)</th>
<th>Twitch amplitude (% resting length)</th>
<th>Maximal shortening velocity (μm/sec)</th>
<th>Maximal shortening velocity/resting cell length</th>
<th>Time to peak shortening (msec)</th>
<th>Relaxation time (msec)</th>
<th>Contraction duration (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>140±5.1</td>
<td>6.0±0.17</td>
<td>165±9.9</td>
<td>1.17±0.103</td>
<td>146±7.8</td>
<td>84±4.8</td>
<td>229±11.4</td>
</tr>
<tr>
<td>HY</td>
<td>145±1.8</td>
<td>7.5±1.02</td>
<td>326±29.8</td>
<td>2.34±0.268</td>
<td>89±5.1</td>
<td>51±8.0</td>
<td>140±5.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Steady-state twitch parameters were measured in myocytes from five euthyroid (EU) and five hyperthyroid (HY) hearts (15 myocytes per heart) when field stimulated at slack length (24/min, 29°C, 1.0 mM [Ca2+]). EU and HY are significantly different (p<0.001) for all parameters except cell length and twitch amplitude (Student’s t test, n=5 for each group).

**Spontaneous SR Calcium Release**

Because of the altered SR function that characterizes hyperthyroid myocardium as well as the alterations in calcium homeostasis,10,13,21,22,27 we hypothesized that the frequency of spontaneous SR calcium release might differ in the euthyroid and hyperthyroid state. Therefore, we measured spontaneous contractile waves as a measure of spontaneous SR calcium release under a variety of conditions in which they have been noted to occur. One such condition in rat myocytes is the absence of electrical stimulation.19 Figure 1 indicates that spontaneous SR calcium release is more likely to occur in unstimulated hyperthyroid myocytes than euthyroid cells.

**The Interaction of Spontaneous SR Calcium Release and Stimulated Twitches**

While rat myocardium manifests spontaneous SR calcium release in the unstimulated state, as described above, this can be suppressed by electrical stimulation.19 After cessation of stimulation, spontaneous SR calcium release then reappears. The time from the last stimulated twitch to the first evidence of spontaneous SR calcium release, or “delay interval,”
is determined by the kinetics of "calcium cycling" within the myocyte. A major determinant of the overall rate of this calcium cycling is the extent of cell calcium loading.\textsuperscript{18,19} Figure 2 shows the results of a representative euthyroid cell during an experiment wherein cell shortening during and after 1 minute of field stimulation at 1 Hz was recorded. Note the appearance of spontaneous SR calcium release after cessation of stimulation. The delay interval, under similar conditions, is markedly reduced in hyperthyroid compared with euthyroid myocytes (19±5.4 vs. 49±12.0 seconds, \(n=5\), \(p<0.05\) by unpaired \(t\) test).

The occurrence of spontaneous SR calcium release during the diastolic interval is associated with a diminished amplitude of the subsequent twitch.\textsuperscript{19} Furthermore, as the time from spontaneous SR calcium release increases, the degree of twitch inhibition diminishes. This is thought to occur due to a depletion of a common SR calcium store by spontaneous SR calcium release and the repletion of the store with time.\textsuperscript{19}

We next determined whether the altered function or enhanced cell calcium loading referred to above that is characteristic of the hyperthyroid state alters the restitution of twitch amplitude after spontaneous SR calcium release. Cells were stimulated at 1/min, and twitch amplitude and the time from the last occurrence of spontaneous SR calcium release to the subsequent twitch were recorded. Figure 3 (top panel) is representative of a euthyroid myocyte with diastolic waves and stimulated twitches indicated. Figure 3 (bottom panel) is aggregate data showing the diminution in twitch amplitude (related to the maximum [rested state] twitch) in a given cell versus the time from the preceding diastolic SR calcium release. Euthyroid myocytes, in accord with previous results, manifest a diminution in amplitude of a test twitch occurring after spontaneous SR calcium release, and there is a gradual recovery of twitch amplitude (a decrease in twitch inhibition) with time. Figure 3 (bottom panel) shows that the same phenomenon occurs in hyperthyroid myocytes but that the time course of twitch recovery is faster. Similar results were obtained in all cells so examined. This increased calcium cycling is in accord with the studies of isolated SR, which show an increased rate of calcium uptake in the hyperthyroid state.\textsuperscript{10,23,25–27}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Histogram of the interwave period of unstimulated euthyroid and hyperthyroid myocytes (15 cells per heart) isolated from five hearts of each type (37 °C, 1.0 mM [Ca\(^{2+}\)]). The interwave period is the inverse of the wave frequency, i.e., the likelihood for a wave to occur. The two distributions are significantly different at \(p<0.01\) by \(\chi^2\) test.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{A representative recording of a euthyroid cell at 37 °C showing rest waves (●), stimulated twitches (○), and the delay interval from the last stimulated twitch until the first spontaneous wave. Note that pacing transiently suppresses the rest waves, which then return after the cessation of pacing.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Recordings and graph showing inhibition of twitch amplitude versus time from the preceding wave (1/min, 37°C, 1.0 mM [Ca\(^{2+}\)]. Top panel: Representative recordings of waves (●) and twitches (○) from a single euthyroid myocyte. Bottom panel: Reduced data from a representative euthyroid (●) and hyperthyroid (○) myocyte. Note that twitch restitution in hyperthyroid myocytes requires less time than euthyroid myocytes (e.g., at 5 seconds after a wave, the hyperthyroid myocyte has its twitch diminished only 10% from its maximal value, but the euthyroid myocyte has 30% inhibition).}
\end{figure}
Measurements were made of twitch amplitudes, dimensions, and calcium release rates, euthyroid and hyperthyroid myocytes demonstrate equal fractional shortening. However, at the lowest frequency of stimulation (6/min), hyperthyroid myocytes shorten less than euthyroid myocytes. The reason for this apparent paradoxical behavior is illustrated in Figure 5, which shows that at low frequencies of stimulation spontaneous SR calcium release occurs more frequently in the diastolic interval in hyperthyroid than in euthyroid myocytes and is associated with a diminution of twitch amplitude.

**Ouabain**

Cardiac glycosides are known to inhibit the sarcolemmal Na⁺,K⁺-ATPase, and the resultant rise in intracellular sodium leads to a rise in intracellular calcium via transsarcolemmal Na-Ca exchange. To determine whether the relative hyperpoperformance of hyperthyroid myocytes at low rates of stimulation (which leads to enhanced SR calcium loading in rat cardiac myocytes) was unique to the experimental paradigm in Figures 4 and 5 or whether it is representative of a general tendency of hyperthyroid myocytes to manifest spontaneous SR calcium release and twitch diminution during conditions that increase intracellular calcium loading, myocytes were superfused with increasing concentrations of the cardiac glycoside ouabain (Figures 6–8).

Figure 6 depicts the fraction of cells that exhibit diastolic SR calcium release at various concentrations of ouabain when electrically stimulated at a rapid (120/min) or a slower (24/min) rate. Note the absence of spontaneous SR calcium release in the absence of ouabain at both frequencies of stimulation in both hyperthyroid and euthyroid myocytes. (Those waves that were present at frequencies of stimulation less than 24/min were overdriven.) As the cells are exposed to increasing concentrations of ouabain, they are more likely to exhibit spontaneous SR calcium release. Figure 6 shows that ouabain, which increases intracellular calcium, is more likely to produce spontaneous SR calcium release in hyperthyroid cells than in euthyroid cells. This suggests that the finding of more frequent spontaneous SR calcium release in hyperthyroid myocytes is a manifestation of increased intracellular calcium in general and not unique to the situation of low stimulation rates (Figures 4 and 5).
thyroid and euthyroid cells (Figure 6). This is best observed in individual cells. Figure 8 depicts the response of a representative hyperthyroid and euthyroid cell to increasing concentrations of ouabain. The maximal twitch amplitude as well as the average amplitude of 10 twitches in the same myocyte at each concentration of ouabain is shown. The concentration of ouabain at which the cell manifested spontaneous SR calcium release is indicated by a horizontal arrow. Note that there is concordance of the maximal twitch and the average of the 10 twitches until the concentration of ouabain is reached where spontaneous SR calcium release occurs. Above this concentration, the average twitch is considerably less than the maximal twitch. The mechanism for this discrepancy is best explained by the concept of twitch restitution, described above, and is illustrated in Figures 3 and 5. When spontaneous SR calcium release occurs, it diminishes the amplitude of the subsequent twitch. The interval between the occurrence of spontaneous SR calcium release and the preceding or subsequent twitch is not precisely regular, and thus arises the variability in twitch amplitude.

Discussion

Validation and Basic Contractile Parameters of Isolated Myocytes

The results show that isolated single myocytes maintain the characteristic twitch properties of intact papillary muscles: the hyperthyroid state results in a reduction of the time to peak shortening, time to 50% relaxation, contraction duration, and an increase in the velocity of shortening. Prior investigators (e.g., Reference 2) have been constrained to measure the velocity of isotonic shortening at varying loads and to extrapolate to what the unloaded veloc-

![Graph showing percent of cells manifesting diastolic waves as a function of ouabain concentration.](image)

**Figure 6.** Graphs showing percent of cells manifesting diastolic waves as a function of ouabain concentration at 37°C and 0.5 mM [Ca²⁺]. Note that, at either pacing frequency, hyperthyroid myocytes (n=6) are more likely than euthyroid myocytes (n=6) to manifest diastolic waves when exposed to various concentrations of ouabain (p<0.5 by Fisher's exact test).

Figure 7 shows that euthyroid cells demonstrate a conventional monotonic increase in twitch amplitude in response to increasing concentrations of glycoside (over the range employed). Twitch amplitude was calculated as the average of 10 consecutive beats in a given myocyte. The cells had been superfused with a given concentration of glycoside for a minimum of 10 minutes, and the average twitch amplitude did not vary by more than 10% with time. The response of hyperthyroid cells is distinctly different. Twitch amplitude increases until it reaches a maximum at approximately 10⁻⁷ M ouabain and then declines. The mechanism for this biphasic response, as well as the large intercell variability among the hyperthyroid cells, is due to different threshold for the occurrence of spontaneous SR calcium release between hyper-

![Dose-response curves of myocytes at 37°C and 0.5 mM [Ca²⁺] to various concentrations of ouabain.](image)

**Figure 7.** Dose-response curves of myocytes at 37°C and 0.5 mM [Ca²⁺] to various concentrations of ouabain at two stimulation rates. Left panel: Actual recordings from representative euthyroid and hyperthyroid myocytes. Diastolic waves (●) occur at lower concentrations of ouabain in hyperthyroid myocytes versus euthyroid myocytes. The biphasic inotropic response to ouabain is evident in the hyperthyroid myocyte, where twitch amplitude is maximum at 10⁻⁷ M ouabain. Right panel: Summary data from euthyroid (n=7) and hyperthyroid (n=6) myocyte (1 myocyte/heart). The ordinate is the twitch amplitude of the cells, normalized in each cell to the maximal amplitude (mean±SEM). Note the biphasic curves for hyperthyroid cells.
lateral myocytes (shortening from slack length), the relative differences between euthyroid and hyperthyroid may be expected to be preserved. Thus, the finding of faster relaxation in hyperthyroid myocytes compared with euthyroid myocytes both confirms prior work in bulk muscle and suggests that the faster relaxation of hyperthyroid rat myocardium is secondary to altered SR function or myosin isoenzyme composition (the latter may affect the rate of calcium dissociation from the myofilaments via an enhanced rate of crossbridge turnover during shortening, leading to a greater extent of shortening deactivation\textsuperscript{19}). Thus, altered loading (both the load imposed by the peripheral vasculature on the myocardium and the load imposed by the process of cardiac hypertrophy on individual myocytes) appears to play a secondary role.

Our data, then, suggest that alterations in contractile properties found in the hyperthyroid state are intrinsic, at least in part, to the myocytes themselves and not merely reflective of alterations in loading conditions, systemic metabolic demands, tissue catecholamine content or release, or catecholamine responsiveness.

### Spontaneous SR Calcium Release

Spontaneous SR calcium release increases with perturbations that are known to increase intracellular calcium. It is possible that the increased frequency of spontaneous SR calcium release that characterizes unstimulated hyperthyroid (versus euthyroid) myocytes is due to an increase in intracellular calcium, or at least calcium in the “releasable pool” of the SR. This increased frequency may be the functional correlate of the increased ability of the hyperthyroid SR to sequester calcium. The increased frequency of spontaneous SR calcium release of hyperthyroid versus euthyroid myocytes seen at low pacing frequencies or ouabain exposure may be a manifestation of enhanced transsarcolemmal calcium influx, the greater number of glycoside receptors,\textsuperscript{10,18,29} or changes in SR calcium uptake.\textsuperscript{10,25–27}

A related study\textsuperscript{24} of calcium flux measurements in a model system of cultured embryonic chick cardiocytes has shown that the hyperthyroid state results in increased transsarcolemmal calcium influx and efflux. In this chick model, thyroxine administration also caused an increase in caffeine-induced efflux of intracellular calcium, suggesting an increase in SR calcium stores. Thus, in this model, the hyperthyroid state alters intracellular calcium dynamics via changes in both the sarcolemma and SR Ca\textsuperscript{2+} transport. Interpretation of these data, however, is confounded by the fact that these cardiocytes contract spontaneously and that the beating rate varies with the thyroid state. Additionally, hyperthyroid-induced changes in the myocardium have been shown to be different in the in situ heart versus a heart that is merely exposed to high concentrations of thyroxine.\textsuperscript{34}

Previous studies have found that inotropic agents, which increase calcium loading, have a paradoxical response in the hyperthyroid state; that is, at high

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**FIGURE 8.** Dose-response curves of individual hyperthyroid and euthyroid myocytes at 37° C and 0.5 mM [Ca\textsuperscript{2+}] to ouabain (mean±SEM). Note the close correlation between average twitch (○) and maximal twitch (●) at concentrations below that which cause diastolic waves (beginning of arrow). Once the waves occur, average twitch amplitude is diminished from the maximum.
concentration, twitch amplitude becomes reduced from its maximum level. The above results suggest that spontaneous SR calcium release may affect the ouabain-induced inotropic response and account for this paradoxical decrease in twitch amplitude. Ouabain causes a rise in intracellular calcium. This increase in intracellular calcium will, up to a point, result in an increase in twitch amplitude. However, once the threshold for spontaneous diastolic SR calcium release (i.e., waves) is reached, the descending portion of the ouabain dose-response curve will soon begin. Reexamination of Figure 6 shows that, at any given concentration of ouabain, hyperthyroid cells are more likely to exhibit SR calcium release than euthyroid cells and, thus, begin the descending portion of the dose-response curve at a lower ouabain concentration. Within the range of concentrations studied, a sufficiently high fraction of hyperthyroid cells will manifest spontaneous SR calcium release, and thus, we observed the biphasic response. There is a suggestion of a biphasic response in euthyroid cells when stimulated at 24/min (Figure 7B), and this is in accord with approximately 40% of these cells manifesting spontaneous SR calcium release at 10\(^{-7}\) M ouabain. Euthyroid cells, when stimulated at 120/min, have a more obvious monotonic response, and few cells have spontaneous SR calcium release under these conditions.

The relatively large variance in Figure 7B may be related to two findings. The spectrum of thresholds for ouabain-induced spontaneous SR calcium release among cells coupled with the spectrum of twitch amplitudes within a given cell (Figure 8) produces a diversity of cellular behavior. It is noteworthy that experiments involving bulk muscle measure the ensemble average of the behavior of individual cells and that this spectrum of response is obscured.

**Ouabain**

Both the euthyroid and hyperthyroid cells respond to ouabain at a lower concentration than is usually said to be the case for in vitro rat systems. There are several possible explanations for this finding. The nature of our isolated cell preparations minimizes diffusion barriers, and thus, for a given bathing drug concentration, the concentration that is actually at the cell surface (i.e., receptor) may be greater than in other model systems. It has previously been demonstrated that perfusion of rat heart with a nominally calcium-free solution increases the sensitivity of Na,K-ATPase to inhibition by digitalis glycosides, and the initial step in the isolation procedure for cardiac myocytes employs a buffer without added calcium ([Ca\(^{2+}\)]\(_{b}\)<50 \(\mu\)M).

In summary, interventions that are known to increase SR calcium loading, such as modest doses of digitalis glycosides or (in rat myocytes) moderate reduction of stimulation frequency (i.e., 1 Hz vs. 2 Hz), increase contractility of both euthyroid and hyperthyroid myocytes. However, interventions that cause a greater increase in intracellular calcium, such as higher doses of digitalis glycosides or low rates of stimulation (in rat), increase contractility in euthyroid myocytes but augment the already increased spontaneous SR calcium release of hyperthyroid myocytes into a range that can limit twitch amplitude. Waves occur between paced beats and are associated with a diminished inotropic response. This is because spontaneous SR calcium release is associated with depletion of the releasable pool of SR calcium. It has been shown in other systems that an increase in SR calcium cycling is associated with an increase in diastolic tone. Thus, interventions that increase intracellular calcium and augment myocardial contractility in the euthyroid state may perturb intracellular calcium homeostasis to such an extent that they do not result in this augmentation in the hyperthyroid heart. This may be an explanation, in part, for the paradoxical hypoperformance of the hyperthyroid heart under certain circumstances.

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