Sarcolemma, Sarcoplasmic Reticulum, and Sarcomeres As Limiting Factors in Force Production in Rat Heart

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Inotropic interventions were compared with respect to their maximum effect on force of contraction in rat myocardium to identify limiting steps in calcium handling. Peak force, sarcomere length, and action potentials were measured in thin ventricular trabeculae. Relevant control conditions were stimulation frequency, 0.2 Hz; [Ca^{2+}]_o 1 mM; [K^+]_o 5 mM; [Na^+]_o 150mM. The inotropic interventions and results were as follows. 1) The interventions of high [Ca^{2+}]_o, low [Na^+]_o, high [K^+]_o, addition of tetraethylammonium chloride, or postextrasystolic potentiation resulted in approximately the same (within 5%) maximum force (F_{max}). Above the respective optimum doses, force declined and aftercontractions were often observed. Combinations of the different interventions never enhanced force to above F_{max}. This suggests that F_{max} is determined by a maximum level of Ca^{2+} in the sarcoplasmic reticulum, above which spontaneous release occurs. 2) Sr^{2+} (10 mM) caused an increase of force to 1.3×F_{max} and lengthening of contraction and action potentials. The force-sarcomere length relation was, then, similar to that in skinned fibers at maximum activation. Hence, 1.3×F_{max} reflects saturation of the sarcomeres. We postulate that a large influx of Sr^{2+} during the long action potential can circumvent the reticulum and activate the sarcomeres directly. When the reticulum was blocked by ryanodine, maximum force of tetanic contractions was about 1.1×F_{max}. This result supports the above conclusions. 3) Isoproterenol increased force to a maximum that was 20% below F_{max} and shortened the contraction. This may be due to a decreased sensitivity of the sarcomeres to Ca^{2+} or to stimulation of the Ca^{2+} pump in the reticulum, that is, an increasing fraction of the released Ca^{2+} is sequestered before it can activate the sarcomeres. Thus, three factors that limit force production were identified, depending on the inotropic stimulus. (Circulation Research 1990;67:913–922)

Force of contraction in heart muscle can be increased by a number of drugs, ions, and stimulus patterns. Naturally, the ultimate maximum force is reached when the contractile filaments are saturated with Ca^{2+} and work at maximum capacity. So far, there is no evidence, however, that this condition occurs in normal, intact heart muscle. On the contrary, indications are that force of contraction in fully activated skinned heart muscle is about 30% greater than maximum force in fibers with intact sarcolemma. Hence, there may be a limiting factor that prevents full activation of the sarcomeres in intact heart muscle. Because the sarcoplasmic reticulum is the main source of activator Ca^{2+}, maximum force may be determined by the maximum capacity of the reticulum to store and release Ca^{2+}. It has been suggested that once Ca^{2+} in the reticulum exceeds a certain level, spontaneous release occurs, and this may curtail the maximum inotropic effect of an intervention. We observed earlier that force in intact trabeculae of rat heart at high [Sr^{2+}], was 30% greater than at high [Ca^{2+}]. Thus, maximum force may be limited by a Ca^{2+}-dependent mechanism that is abolished or circumvented by Sr^{2+}. We tested this hypothesis by measuring the maximum effect of various interventions in intact trabeculae. We used laser diffraction to measure sarcomere length and microelectrodes to measure membrane potential, that is, methods that
give information about organelle activity while having no influence on their properties.

Materials and Methods

Details of the preparation and methods have been described earlier. In brief, long (2.5–4-mm) and thin (0.05–0.15-mm) free-running trabeculae were dissected from the right ventricle of rat heart and suspended in a 0.5-ml chamber between a force transducer (type AE801, Mikro-Elektronik, Horten, Norway) and a length adjustment device. The muscle was stretched to a length at which passive force was about 2% of the active force at 2.5 mM [Ca\(^{2+}\)]\(_o\) and stimulated at 1 Hz for 1 hour before the experiment was begun.

The composition of standard solution was (in mM): NaCl 120, KCl 5, MgCl\(_2\) 1.2, CaCl\(_2\) 1.0, NaH\(_2\)PO\(_4\) 2, Na\(_2\)SO\(_4\) 1.2, NaHCO\(_3\) 27, and glucose 10. The solution was recirculated via a 0.5-l reservoir in which it was gassed with 95% O\(_2\) and 5% CO\(_2\); PO\(_2\) was 590–650 mm Hg, PCO\(_2\) was 39–45 mm Hg, and pH was 7.35–7.45. Flow through the muscle chamber was 10 ml/min, and temperature was 27±0.3°C. The calcium and strontium concentrations in the superfusion solution ([Ca\(^{2+}\)]\(_o\) and [Sr\(^{2+}\)]\(_o\)) were varied by adding amounts of 1 M stock solutions of the chloride salts to standard or Ca\(^{2+}\)-free solution. Low [Na\(^{+}\)]\(_o\) was obtained by substitution of 15–90 mM NaCl by 15–90 mM LiCl. In experiments with isoproterenol, a calibrated syringe and infuser pump were used to inject the drug into the Tyrode’s solution just before the inlet of the muscle bath to prevent time-dependent effects of oxidation. The Tyrode’s solution was not recirculated during the isoproterenol experiments.

Ryanodine-treated muscles were used to study tetanic contractions at high [Ca\(^{2+}\)]\(_o\). To prevent precipitation, NaHCO\(_3\) and Na\(_2\)HPO\(_4\) were replaced with 20 mM NaCl and 10 mM HEPES. The pH was adjusted with NaOH. The solutions were equilibrated with 100% O\(_2\). Contractures were induced with Tyrode’s solution with the following modifications: 60–120 mM NaCl was replaced by LiCl, [KCl] was increased to 20 mM, and 10 mM caffeine was added.

Sarcomere length was measured with laser diffraction, as described earlier. Briefly, the parallel sarcomeres act as an optical grating, and the incident laser beam is split into a zero- and multiple higher-order bands. The spacing between the bands is a measure of sarcomere length. The first-order band was scanned twice per millisecond by a photodiode array. Sarcomere length was computed electronically from the scans. Calibration was performed with test gratings before each experiment.

Transmembrane potential was measured by means of flexible glass microelectrodes. The resistance of the electrodes (filled with 3 M KCl) was 60–100 MΩ, as measured in the muscle chamber.

Stimulus pulses of 3-msec duration and 20% above threshold strength were derived from a programmable pulse generator via a stimulus isolator and two platinum wire electrodes. The frequency of stimulation was 0.2 Hz during all experiments; however, postextrasystolic potentiation was induced by interposing trains of 1–100 intervals of 0.25 second (see Figure 1).

Results

Influence of [Ca\(^{2+}\)]\(_o\)

To determine the peak force-[Ca\(^{2+}\)]\(_o\) relation, the [Ca\(^{2+}\)]\(_o\), was first reduced to 0.3 mM and subsequently increased in several steps, at intervals of 5 minutes, to a final concentration of 5–10 mM. The result was a sigmoid relation, and maximum force (F\(_{max}\)) occurred at about 3 mM. Above 3 mM, force declined (e.g., Figures 2, 4, and 8) and aftercontractions were observed. F\(_{max}\) determined at optimum [Ca\(^{2+}\)]\(_o\) was used as a reference; that is, the maximum effects of all other interventions were compared with that value.

Postextrasystolic Potentiation

The optimum interval to induce postextrasystolic potentiation in rat myocardium is 0.25 second, and short trains of 0.25-second intervals in low [Ca\(^{2+}\)]\(_o\) potentiated force severalfold until a maximum effect, which is indicated as “saturation” (Figure 1). The figure also shows that increasing the number of extrasystoles above the optimum caused a small decrease of peak force. The latter effect was associated with aftercontractions. The number of extrasystoles that caused saturation decreased with increasing [Ca\(^{2+}\)]\(_o\) (Figure 1).

It should be noted that aftercontractions were well visible through the microscope but generally not in the record of force. This follows from the fact that the aftercontraction was a propagated wave. Thus, at a given moment only a small fraction of the muscle contracted; the rest was passively stretched. Hence, external force was negligible.

We tested whether saturation corresponded with a unique, maximum value of force or varied with [Ca\(^{2+}\)]\(_o\) or steady-state force. The procedure shown in Figure 1 was used to determine maximum potentiation, and Figure 2 demonstrates that, except for a few points at very low steady-state force and [Ca\(^{2+}\)]\(_o\), the maximally potentiated beats were within a few percent of F\(_{max}\) that is, independent of steady-state force and [Ca\(^{2+}\)]\(_o\).

Low [Na\(^{+}\)]\(_o\), and High [K\(^{+}\)]\(_o\)

Both low [Na\(^{+}\)]\(_o\), and elevated [K\(^{+}\)]\(_o\), (depolarization of the membrane) inhibit extrusion of Ca\(^{2+}\) via the Na\(^{+}\)/Ca\(^{2+}\) exchanger. This causes intracellular accumulation of Ca\(^{2+}\) and enhanced force. In addition, increased [K\(^{+}\)]\(_o\) lengthens the action potential in heart muscle of rat (see Figure 4C).

In each preparation we first determined F\(_{max}\) by means of postextrasystolic potentiation and usually by varying [Ca\(^{2+}\)]\(_o\). Reduction of [Na\(^{+}\)]\(_o\), in the presence of 1 mM Ca\(^{2+}\), led to enhanced force and shortening of the action potential, as has been shown earlier. The maximum inotropic effect was found at about 75 mM [Na\(^{+}\)]\(_o\), and force was then (1.04±0.04)×F\(_{max}\) (n=5). Reduction of [Na\(^{+}\)]\(_o\), below 75
mM caused aftercontractions and a decline in force (Figure 3).

The resting membrane potential became more positive after elevation of $[K^+]_o$, while the upstroke of the action potential became slower and action potential duration at 50% of the amplitude increased. To compensate for the decreased excitability at 15–20 mM $[K^+]_o$, the strength and duration of the stimulus had to be increased threefold. Force increased with $[K^+]_o$ to a maximum at about 15 mM and remained constant (Figure 4A) or decreased slightly at higher concentrations. Aftercontractions were not distinguishable in the recordings of force. Maximum peak force obtained with high $[K^+]_o$ was always equal to or slightly less than $F_{\text{max}}$ obtained with postextrasystolic potentiation or optimum $[Ca^{2+}]_o$. The mean was $(0.95\pm0.05)\times F_{\text{max}}$ ($n=5$).

We tested whether the combination of high $[K^+]_o$ and high $[Ca^{2+}]_o$ or low $[Na^+]_o$ and high $[Ca^{2+}]_o$ could enhance force to above $F_{\text{max}}$. This was not the case, and when applied at optimum $[Ca^{2+}]_o$ elevation of $[K^+]_o$ (Figure 4B) or reduction of $[Na^+]_o$ caused aftercontractions and a decline in force. Elevation of $[K^+]_o$ always lengthened the action potential, also at optimum $[Ca^{2+}]_o$ (Figure 4C).

**Influence of Tetraethylammonium**

Tetraethylammonium (TEA) blocks the $K^+$ outward currents $I_w$ and $I_{Kt}$,12,13 which explains the marked lengthening of the action potential (Figures 5A and 5B). When added to standard solution (1 mM $[Ca^{2+}]_o$), TEA enhanced force to a maximum of $(0.96\pm0.07)\times F_{\text{max}}$ ($n=3$). The optimum concentration was 5–10 mM; at higher concentrations force declined and aftercontractions were observed. Action potential duration continued to increase with $[\text{TEA}]_o$. In the presence of 2.5 mM $[Ca^{2+}]_o$, when force was already close to $F_{\text{max}}$, TEA induced only a small increase in force, or a decrease (Figure 5C). Action potential duration always increased with $[\text{TEA}]_o$ (Figures 5A and 5B).

**Influence of Isoproterenol**

Commonly observed effects of $\beta$-receptor agonists are enhanced force, shortened twitch duration, and lengthened action potential duration.14-16 We found the same effects in the rat trabeculae. In the experiments with this drug we first determined $F_{\text{max}}$ by variation of $[Ca^{2+}]_o$ and postextrasystolic potentiation. Subsequently, an isoproterenol dose-response relation was measured in control solution. Above the threshold concentration of 10 nM, peak force began to increase and maximum force was found at about 1

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Maximum effect of interposed extrasystoles. Upper panel: At 0.5 mM $[Ca^{2+}]_o$, maximum potentiation (indicated as saturation) was obtained with a train of 75 extrasystoles (0.25-second intervals). In this preparation peak force ($F_{F_{\text{max}}}$) was relatively low (diamonds in Figure 2); in other preparations a smaller number of extrasystoles was sufficient to induce saturation. Lower panel: At 2 mM $[Ca^{2+}]_o$ the maximum effect occurred after 20 extrasystoles. These two recordings were from the same preparation as represented by the diamonds in Figure 2. $F_{\text{max}}$, maximum force at optimum $[Ca^{2+}]_o$.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Dependence of normalized peak force ($F/F_{\text{max}}$) on $[Ca^{2+}]_o$ in five trabeculae. Open symbols represent steady-state force measured 3 minutes after each increment in concentration. At each concentration, maximum postextrasystolic potentiation (solid symbols) was determined with the protocol shown in Figure 1.
FIGURE 3. Maximum effect of low \([\text{Na}^+]_o\) (Li\(^+\) substitution) on peak force (\(F/F_{\text{max}}\)) compared with maximum postextrasystolic potentiation. The recording at high paper speed shows that stimulated contractions at 60 mM \([\text{Na}^+]_o\) were followed by aftercontractions. \(F_{\text{max}}\): maximum force at optimum \([\text{Ca}^{2+}]_o\).

Influence of Ryanodine and Caffeine

Application of 5 \mu M ryanodine reduced steady-state force (at 1 mM \([\text{Ca}^{2+}]_o\), 0.2 Hz) to 5\% and completely abolished postextrasystolic potentiation. These effects are consistent with the general assumption that this drug abolishes the sarcoplasmic reticulum as a functional \text{Ca}^{2+}\text{ store}\text{.}^{17-19}

Rapid stimulation induced tetanic contractions that peaked within a few seconds and then decreased. The most effective frequency was 6–8 Hz, at which the amplitude of the tetanus was greater than that of single twitches (0.2 Hz) and increased with \([\text{Ca}^{2+}]_o\). When we switched from bicarbonate to

\[\text{K}^+ \text{ in mM: } 5, 7, 9, 11, 13, 15, 18, 21\]

\[\text{Ca}^{2+} \text{ in mM: } 1, 1.5, 2.0, 2.5\]

Panel A: Maximum effect of postextrasystolic potentiation and of high \([\text{K}^+]_o\) on force. Panel B: Maximum effect of postextrasystolic potentiation and of high \([\text{Ca}^{2+}]_o\) on force. Note that, in the presence of 2.5 mM \([\text{Ca}^{2+}]_o\), extrasystoles were followed by depressed contractions (arrows above the recording) and increased \([\text{K}^+]_o\), also depressed contraction. Panel C: Action potentials at 5 and 11 mM \text{K}^+ in the presence of 2.5 mM \([\text{Ca}^{2+}]_o\). Recordings in panels A, B, and C were from one preparation. \(F/F_{\text{max}}\): peak force. \(F_{\text{max}}\): maximum force at optimum \([\text{Ca}^{2+}]_o\).
HEPES-buffered solution (1 mM [Ca$^{2+}$]$_o$ in both), a transient increase in force was observed that lasted about 15 minutes and was followed by a decrease in force to a lower level. An example of tetanic contractions after ryanodine treatment is shown in Figure 7. In four of five preparations, the maximum tetanic force at 8–15 mM Ca$^{2+}$ was greater than F$_{max}$ and the average was (1.10±0.17)×F$_{max}$ ($n=5$). It should also be noted that diastolic force was 11±8% of F$_{max}$ in ryanodine-treated muscles at 8 mM Ca$^{2+}$, whereas diastolic force induced by the other interventions never exceeded 2%.

Caffeine (5–10 mM) reduced force to 50% and abolished postextrasystolic potentiation, which is consistent with the hypothesis that caffeine inhibits reticulum function.$^{19-21}$ The action potential was lengthened by about 100%, probably as a result of intracellular cyclic AMP accumulation and cyclic AMP–induced enhancement of the inward Ca$^{2+}$ current (I$_{Ca}$).$^{22}$ During rapid stimulation, a tetanic contraction with variable amplitude developed in the presence of caffeine, and without electrical stimulation a large contracture developed when the muscle was superfused with a caffeine solution at a low [Na$^+$]$_o$ and high [K$^+$]$_o$. The amplitude of these contractures was rather variable, (0.95±0.21)×F$_{max}$ ($n=5$).

**Influence of Sr$^{2+}$**

Strontium was of special interest because we found earlier$^7$ that it could enhance peak force substantially above F$_{max}$. Because replacement of all extracellular Ca$^{2+}$ by Sr$^{2+}$ led to rapid deterioration of the muscle (irreversible depolarization and visible changes in cell structure), we tested the effect of Sr$^{2+}$ in standard solution (1 mM [Ca$^{2+}$]$_o$). As shown in Figure 8 (open symbols), 1–5 mM [Sr$^{2+}$]$_o$ was less effective than Ca$^{2+}$ to increase force, but at higher concentrations force reached a plateau that was 20–40% greater than F$_{max}$ obtained with postextrasystolic potentiation or high [Ca$^{2+}$]$_o$ in the absence of Sr$^{2+}$.

Besides an increase of peak force, addition of Ca$^{2+}$ to control solution also led to increase of contraction duration (+30% at 3 mM Ca$^{2+}$) and shortening of the action potential (−25% at 3 mM Ca$^{2+}$).$^7$ When we added Sr$^{2+}$ instead of Ca$^{2+}$, the prolongation of the contraction (+150%) was more pronounced, and the action potential was prolonged (+200%) (Figure 9A). As a consequence of this slow relaxation, rapid stimulation caused fusion and summation of contractions. At 10–19 mM [Sr$^{2+}$]$_o$, 4 Hz stimulation induced a tetanic contraction that never exceeded the amplitude of single twitches and was followed by a series of aftercontractions (Figure 9B). Such tetanic contractions did not develop at high [Ca$^{2+}$]$_o$ in the absence of Sr$^{2+}$.

**Force–Sarcomere Length Relation**

At control length of the muscle (2% passive force), sarcomere length was 2.2–2.3 μm. During the contractions, however, sarcomere length in the central region
shortened to about 1.9 μm at the expense of stretch in the damaged ends, as reported earlier. Every 10th beat, muscle length was changed for one beat so that active sarcomere length at the moment of peak contraction varied from 1.6 to 2.2 μm. In between the test beats the muscle was held at control length. At 0.8 mM \([Ca^{2+}]_0\), force increased in a linear fashion with sarcomere length (triangles in Figure 10). At 2.5 mM the relation was nonlinear (circles in Figure 10), and at 5 mM a similar curve was obtained, but force was smaller than at 2.5 mM at any sarcomere length (solid triangles in Figure 10), which is consistent with Figures 2 and 8. At 9–19 mM \([Sr^{2+}]_0\) (1 mM \([Ca^{2+}]_0\), present), however, peak force increased to values well above those measured at optimum \([Ca^{2+}]_0\) and as shown in Figure 10, the effect of Sr was greatest at short sarcomere length. Thus, the force-sarcomere length curve at high \([Sr^{2+}]_0\) is remarkably similar to

**Figure 7.** Tetanic contractions in a ryanodine-treated trabecula. Maximum postextrasystolic potentiation was recorded in standard solution with bicarbonate as the pH buffer (left panel) and subsequently after replacement of the bicarbonate with HEPES (middle panel). \([Ca^{2+}]_0\) was 1 mM in both solutions. The trabecula was exposed to 5 μM ryanodine for 20 minutes, and after that tetanic contractions were induced with 6 Hz stimulation for 10 seconds at increasing \([Ca^{2+}]_0\) (right panels). \(F/F_{max}\) peak force.

**Figure 8.** Dependence of steady-state peak force (\(F/F_{max}\)) on \([Ca^{2+}]_0\) (solid symbols) and \([Sr^{2+}]_0\) (in the presence of 1 mM \([Ca^{2+}]_0\), open symbols). Each pair of curves represents one preparation. \(F_{max}\) is the maximum force recorded at optimum \([Ca^{2+}]_0\), and data were normalized to that value.

**Figure 9.** Panel A: Effect of 19 mM \([Sr^{2+}]_0\) (in the presence of 1 mM \([Ca^{2+}]_0\)) on contraction and action potential. The unlabeled action potential and contraction were recorded at 1 mM \([Ca^{2+}]_0\). Panel B: In the presence of 1 mM \([Ca^{2+}]_0\), and 19 mM \([Sr^{2+}]_0\), rapid stimulation (4 Hz) caused a decrease in action potential amplitude and incomplete repolarization (top tracing) and a tetanic contraction (middle tracing). The bottom tracing shows the stimulus pulses. The arrows point at afterdepolarizations (top tracing) and aftercontractions (middle tracing). \(F/F_{max}\) peak force. \(F_{max}\) maximum force at optimum \([Ca^{2+}]_0\).
It should be noted that at a sarcomere length above 2.2 μm and below 1.8 μm, passive elastic forces occur, which contribute to the externally measured force. At slack length (about 1.9 μm) the passive forces cancel each other.\textsuperscript{23,24} In our experiments, the sarcomere length at peak contraction was about 1.9 μm, so that passive forces can be ignored and external force was equal to the active force generated by the sarcomeres.

Peak force of contraction is assumed to be proportional to the amount of activator Ca\textsuperscript{2+} available in the sarcomplasm. This amount is determined mainly by transport mechanisms in the sarcolemma (I\textsubscript{Ca}, Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange) and in the sarcoplasmic reticulum (Ca\textsuperscript{2+} release channels, Ca\textsuperscript{2+} pump). On the time scale of a contraction, the role of the mitochondria and of the Ca\textsuperscript{2+} pump in the sarcomplasm is probably minor.\textsuperscript{15,25-27} Thus, the limiting factors are expected to be located in the sarcolemma, the sarcoplasmic reticulum, or the contractile filaments.

**Sarcolemma**

The primary effects of increased [Ca\textsuperscript{2+}]\textsubscript{o}, K\textsuperscript{+} depolarization, TEA, and reduced [Na\textsuperscript{+}]\textsubscript{o} are at the level of the sarcolemma: High [Ca\textsuperscript{2+}]\textsubscript{o} enhances Ca\textsuperscript{2+} influx via I\textsubscript{Ca}\textsuperscript{10,28} and via the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger.\textsuperscript{10,11} The ensuing rise in intracellular Ca\textsuperscript{2+}, probably stored in the sarcoplasmic reticulum, explains the observed enhancement of peak force. Low [Na\textsuperscript{+}]\textsubscript{o} and K\textsuperscript{+} depolarization inhibit Ca\textsuperscript{2+} extrusion via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange.\textsuperscript{10,11} Low [Na\textsuperscript{+}]\textsubscript{o} may influence the intracellular pH via the Na\textsuperscript{+}/H\textsuperscript{+} exchanger, but since [Na\textsuperscript{+}]\textsubscript{o} was reduced by only 10–60% and Li\textsuperscript{+} was the substitute, the effect on pH\textsubscript{e} is expected to be negligible.\textsuperscript{29,30}

Finally, TEA blocks the K\textsuperscript{+} outward currents, I\textsubscript{to} and I\textsubscript{K12,13} that are prominent in rat heart and responsible for the short duration of the action potential. Therefore, TEA lengthened the action potential and presumably allowed for a longer-lasting, and hence a greater total, influx of Ca\textsuperscript{2+}, which explains the associated increase in peak force. It is remarkable that despite their different mode of action, all interventions at the level of the sarcolemma led to approximately the same (within±5%) maximum force (Figures 3–5). Furthermore, in the case of high [Ca\textsuperscript{2+}]\textsubscript{o} and low [Na\textsuperscript{+}]\textsubscript{o}, the action potential was slightly shortened,\textsuperscript{7,9} whereas action potential duration continued to increase even when peak force declined with further addition of K\textsuperscript{+} or TEA (Figures 4 and 5). Thus, it appears that the F\textsubscript{max} was not related to action potential duration and was almost independent of the type of intervention on the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (influx facilitation with high [Ca\textsuperscript{2+}]\textsubscript{o}, inhibition of efflux with low [Na\textsuperscript{+}]\textsubscript{o}). It is concluded that mechanisms in the sarcolemma (i.e., the action potential, I\textsubscript{Ca}, and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange) may be responsible for the ±5% variation in F\textsubscript{max}. Apparently, the main limiting factor is in a later step in excitation-contraction coupling.

that reported for skinned myocytes\textsuperscript{1} and skinned trabeculae\textsuperscript{2} of rat heart.

**Discussion**

This study shows that high [Ca\textsuperscript{2+}]\textsubscript{o}, low [Na\textsuperscript{+}]\textsubscript{o}, K\textsuperscript{+} depolarization, TEA, and postextrasystolic potentiation led to the same (within±5%) maximum force (F\textsubscript{max}) in trabeculae of rat heart. This suggests a common limiting factor or saturation of a single mechanism. On the other hand, a value of 1.3×F\textsubscript{max} was obtained with high [Sr\textsuperscript{2+}]\textsubscript{o}, whereas force did not increase above 0.8×F\textsubscript{max} after β-adrenoceptor stimulation. Hence, maximum force assumes different values depending on conditions, indicating the presence of several limiting factors.
**Sarcoplasmic Reticulum**

**Postextrasystolic potentiation.** A simple method to test the capacity of the reticulum is postextrasystolic potentiation. The current explanation for this phenomenon is as follows,\(^\text{15,31,32}\); Recovery of the release mechanism in the reticulum is slow, and therefore, an extra stimulus after a very short interval elicits the release of only a small amount of Ca\(^{2+}\), and the extrasystole is small. The influx of Ca\(^{2+}\) via ICa, however, is enhanced during the extrasystole.\(^\text{7,33}\) This combination of reduced release and enhanced influx causes accumulation of extra Ca\(^{2+}\) in the reticulum and potentiation of the next beat after a normal interval. Thus, maximum potentiation is probably a good indicator for the capacity of the reticulum to accumulate Ca\(^{2+}\). The maximum force obtained with this method was remarkably similar to that obtained with optimum [Ca\(^{2+}\)]\(_o\), [Na\(^+\)]\(_o\), [K\(^+\)]\(_o\), and [TEA], (Figures 2–5). Hence, in all four cases the limiting factor was probably the capacity of the reticulum, or as proposed previously,\(^\text{4,6}\) spontaneous release occurs when the accumulated Ca\(^{2+}\) exceeds a certain level. Because replenishment of the releasable Ca\(^{2+}\) store takes time, the amplitude of stimulated contractions is reduced by the preceding spontaneous releases. In accordance with this hypothesis, aftercontractions (Figure 3) and a decline in force (Figures 2–5) occurred at supramaximal Ca\(^{2+}\) loading.

**Influence of Sr\(^{2+}\).** Sr\(^{2+}\) can replace Ca\(^{2+}\) in at least two mechanisms. First, Sr\(^{2+}\) serves as a charge carrier for the current through the Ca\(^{2+}\) channels in the sarcolemma (ICa) but slows the inactivation of this current.\(^\text{18,28,34}\) This explains the prolonged action potential (Figure 9A). Second, Sr\(^{2+}\) is a potent activator of sarcomere contraction,\(^\text{35,36}\) with the same affinity for the contractile system as Ca\(^{2+}\),\(^\text{37}\) which in combination with the prolonged action potential explains enhanced force. On the other hand, there is evidence that Sr\(^{2+}\) is not stored in the sarcoplasmic reticulum. First, when external Ca\(^{2+}\) was replaced by Sr\(^{2+}\), peak force was delayed and relaxation was slowed (Figure 9A). Such slow contractions may occur under various conditions and indicate that part of the contraction is due to direct activation by Sr\(^{2+}\) (or Ca\(^{2+}\)) influx and not to release from the sarcoplasmic reticulum.\(^\text{25,27,38}\) Furthermore, ryanodine, which inhibits Ca\(^{2+}\) channels in the sarcoplasmic reticulum, greatly reduces contraction in the presence of Ca\(^{2+}\) but not when Ca\(^{2+}\) has been replaced by Sr\(^{2+}\).\(^\text{18,39}\) Thus, there is reason to assume that Sr\(^{2+}\) is not transported by the sarcoplasmic reticulum and that, in the presence of 1 mM Ca\(^{2+}\), activation of the sarcomeres occurs via two parallel paths: release of Ca\(^{2+}\) from the sarcoplasmic reticulum and influx of Sr\(^{2+}\) from the extracellular space. It should be noted that Sr\(^{2+}\) did not block Ca\(^{2+}\) transport by the sarcoplasmic reticulum, since the early component of contraction was not abolished, and rapid stimulation induced aftercontractions (Figure 9B).

We conclude that F_{max} is determined by the capacity of the sarcoplasmic reticulum, and force can exceed F_{max} (Figures 8 and 10) because Sr\(^{2+}\) circumvents this limiting factor.

**Caffeine and ryanodine.** These two drugs caused slowing of twitch relaxation and abolished postextrasystolic potentiation, indicating that the reticulum was effectively abolished as a functional Ca\(^{2+}\) store. Caffeine, however, has multiple effects, and recent reports suggest that it may affect the sensitivity of the sarcomeres to Ca\(^{2+}\).\(^\text{19,21}\) The variable amplitude of contractions and tetanic contractions obtained with caffeine (see “Results”) may be related to the complexity of the effects and precludes firm conclusions in the present context.

Ryanodine suppresses reticulum function in a much more selective way than caffeine does.\(^\text{18,19,40}\) Therefore, after ryanodine treatment, activation of the sarcomeres presumably depends entirely on Ca\(^{2+}\) influx from the extracellular space. The slow relaxation causes summation of contractions (tetanic contractions) at high frequencies of stimulation and [Ca\(^{2+}\)]\(_o\), which is expected to lead to saturation of the sarcomeres.\(^\text{40}\) Our results partly support this idea. In HEPES-buffered solution with 8–12 mM [Ca\(^{2+}\)]\(_o\), maximum force of tetani was (1.1±0.17)×F_{max}, that is, greater than the maximum effect of various other interventions (e.g., Figure 7) but some 20% lower than the maximum at high [Sr\(^{2+}\)]\(_o\), in bicarbonate-buffered solution. Two types of complications may be mentioned. 1) The transition to HEPES-containing solution led to a transient increase in force, which was presumably due to a transient increase in pH, and the associated increase in the sensitivity of the sarcomeres to Ca\(^{2+}\).\(^\text{41}\) Steady-state pH, was probably normal, however, and tests with [Ca\(^{2+}\)]\(_o\), and postextrasystolic potentiation showed that F_{max} was unchanged (Figure 7). 2) Ryanodine-treated muscles always developed substantial diastolic force, which may have imposed an extra load on energy metabolism and caused accumulation of metabolites and acidosis. Furthermore, the continuous elevation of intracellular [Ca\(^{2+}\)] probably inactivates ICa. This second group of complications is supported by the observations that the tetanic contraction reached a peak within a few seconds and then decreased, and in one preparation a steep increase in diastolic force at 12 mM [Ca\(^{2+}\)]\(_o\) was accompanied by a decrease in the amplitude of the tetanic contraction. Nevertheless, the ryanodine results support our hypothesis that force can exceed F_{max} if the reticulum is abolished as a limiting factor.

**Isoproterenol.** Stimulation of β-adrenoceptors leads 1) to enhanced ICa,\(^\text{22}\) which explains the lengthened action potential; 2) to faster sequestration of Ca\(^{2+}\) by the reticulum, which accounts for shortening of twitch duration;\(^\text{16,42}\); and 3) to a decrease in the sensitivity of the sarcomeres to Ca\(^{2+}\).\(^\text{43,44}\) Enhanced Ca\(^{2+}\) uptake by the reticulum implies that a smaller fraction of the released Ca\(^{2+}\) is extruded from the cell via Na\(^+\)/Ca\(^{2+}\) exchange because the two mechanisms
compete. Both the reduced loss of Ca\textsuperscript{2+} per beat and the increased influx via I\textsubscript{Ca} explain the positive inotropic effect of isoproterenol.

We found that maximum force at the optimum [isoproterenol], was 20% below the F\textsubscript{max} obtained with optimum [Ca\textsuperscript{2+}], and various other means. This was not simply due to saturation of the β-receptors because the action potential duration at 50% of the amplitude continued to increase with isoproterenol concentration. Rather, the drug introduced a new, lower maximum level of force, and at optimum [isoproterenol], postextrasystolic potentiation (Figure 6) and elevation of [Ca\textsuperscript{2+}], (not shown) failed to enhance force above 0.8×F\textsubscript{max}. This remarkable finding can be explained from rapid uptake of Ca\textsuperscript{2+} by the sarcoplasmic reticulum or reduced sensitivity of the sarcomeres to Ca\textsuperscript{2+}. The first explanation implies that the Ca\textsuperscript{2+} pump in the reticulum, when stimulated by isoproterenol, sequesters a fraction of the released Ca\textsuperscript{2+} before it reaches the sarcomeres. According to the second explanation, the same amount of Ca\textsuperscript{2+} will be less effective when the sensitivity of the sarcomeres is reduced. Therefore, if the maximum amount of releasable Ca\textsuperscript{2+} is constant, both mechanisms decrease the associated maximum force to below F\textsubscript{max}.

**Sarcomeres**

The force produced at a given level of intracellular Ca\textsuperscript{2+} (and Sr\textsuperscript{2+}) is determined by the affinity of the sarcomeres to Ca\textsuperscript{2+} (and Sr\textsuperscript{2+}) and on the maximum effect of saturation. There is evidence for effects of β-adrenoceptor stimulation on sarcomere affinity, probably via cyclic AMP–dependent phosphorylation of the inhibitory component of troponin.43,44 This may have contributed to the decrease in maximum force at optimum [Ca\textsuperscript{2+}], and postextrasystolic potentiation (Figure 6). Nevertheless, the enhanced relaxation of both force and intracellular Ca\textsuperscript{2+} transient suggests a prominent role of enhanced sequestration by the reticulum in the reduction of maximum force.

Caffeine may affect both properties of the sarcomeres: it increases the affinity but reduces maximum force at saturating levels of Ca\textsuperscript{2+}.19,21 These complications, in addition to the effects on reticulum function, may explain the variability of the caffeine results but render the interpretation difficult.

Ryanodine has no direct influence on the sarcomeres so that its primary effect mainly, or exclusively, concerns abolition of the reticulum as a limiting step in Ca\textsuperscript{2+} availability. Thus, maximum force in ryanodine-treated muscles may reflect Ca\textsuperscript{2+} saturation of unchanged sarcomeres. As argued above, the extreme rate of energy turnover during the tetanic contractions, however, may have caused intracellular acidosis and inorganic phosphate accumulation, which is known to depress force production.45,46 This might have influenced the results, although we used thin trabeculae in which diffusion of metabolites is normally adequate.47 As a consequence, tetanic contractions may lead to underestimation of force in maximally activated sarcomeres.

**Force–sarcomere length relations.** The force–sarcomere length relations in Figure 10 at different [Ca\textsuperscript{2+}],s are similar to those reported by ter Keurs et al\textsuperscript{8} and Gordon and Pollack.29 It has been proposed earlier that F\textsubscript{max} in intact myocardium corresponds to some 70% saturation of the sarcomeres because greater force could be induced in skinned myocytes\textsuperscript{1} and skinned trabeculae\textsuperscript{2} of rat heart. According to percentages, the difference in maximum force between skinned and intact preparations increased at shorter sarcomere lengths, and Figure 10 shows the same: in the presence of Sr\textsuperscript{2+}, maximum force increased by 15% at 2.2 μm and by more than 100% at 1.6 μm. This obviates the possible criticism that the greater force in skinned preparations could be an artifact of the skinnning procedure and supports Fabiato’s hypothesis that under “normal” conditions saturation of the sarcomeres does not occur in intact preparations. The exception is that abolition of the reticulum as a limiting factor apparently does allow for full activation of the sarcomeres, the ultimate limit of force production. It should be noted that saturation with Sr\textsuperscript{2+} caused 10% greater force in skinned fibers than did saturation with Ca\textsuperscript{2+}.35 We conclude that maximum Ca\textsuperscript{2+}-activated force is between the values obtained with ryanodine and Sr\textsuperscript{2+}, that is, between 1.1 and 1.3 of F\textsubscript{max}.

In conclusion, F\textsubscript{max} obtained at optimum [Ca\textsuperscript{2+}], [Na\textsuperscript{+}], [K\textsuperscript{+}], [TEA\textsubscript{+}], and postextrasystolic potentiation was shown to depend mainly on the capacity of the reticulum to store Ca\textsuperscript{2+}. Maximum activation of the sarcomeres was possible when the reticulum was abolished as a limiting factor with high [Sr\textsuperscript{2+}], and ryanodine. The force–sarcomere length relation was then similar to that in skinned fibers at maximal Ca\textsuperscript{2+} activation. The isoproterenol results suggest that a reduced affinity of the sarcomeres to Ca\textsuperscript{2+} and rapid uptake of Ca\textsuperscript{2+} by the reticulum decreased the contractile response to the maximum amount of released Ca\textsuperscript{2+}.

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