The effects of catecholamines on tension reactivation in cardiac muscle

By Y. Shimoni†

Department of Physiology, Hebrew University Hadassah Medical School, Jerusalem, Israel

(Communicated by D. Noble, F.R.S. — Received 29 October 1986)

The effects of adrenaline and the β-agonist isoprenaline on the time course of tension reactivation were studied in several cardiac tissues. The aim of the study was to assess whether experimental evidence can be found for a role of the sarcoplasmic reticulum in the reactivation of tension. It was assumed that calcium recycles between different parts of the reticulum, and that this recycling may affect tension repriming. Isoprenaline was assumed to enhance such recycling by increasing the uptake of calcium, following its release during a preceding contraction.

Isoprenaline (in the range of 40 nM to 4 μM) was found to enhance tension repriming in adult guinea pig atria. However, in adult rat atria, isoprenaline often gave a complex effect, with a smaller degree of repriming at short intervals, and enhanced repriming at longer intervals. This was thought to reflect the balance between the enhancing effect of the drug on calcium recycling and an augmented release from the sarcoplasmic reticulum (SR).

In striking contrast, there was no effect of isoprenaline on tension repriming in neonatal guinea pig atria and a retardation in neonatal rat atria. This was interpreted as reflecting the lack of a sarcoplasmic network in the neonatal tissue. The effects of isoprenaline on tension repriming in the frog atrium (which also has a sparse sarcoplasmic reticulum network) were also found to be complex: low concentrations (40 nM) enhanced the process, and high concentrations (0.4 μM) retarded it. Intermediate levels often produced a 'crossover' effect: more reactivation at short intervals, and less at long intervals.

The interpretation of these results was that there are two processes which interact to determine the amount of tension produced at short intervals after each contraction: the basal reactivation process and some augmenting mechanism superimposed on it. This mechanism is probably related to other behavioural features of cardiac muscle, such as rate-dependent increases in membrane calcium currents. It is relevant mainly in those cases where tension repriming depends on membrane calcium currents.

Further experiments (in the frog atrium) with elevated calcium and with the α-adrenergic agonist phenylephrine (both of which slowed down the reactivation process) also support this idea. These agents elevate internal calcium levels, and presumably saturate the augmenting mech-

† Present address: Department of Medical Physiology, University of Calgary Health Sciences Centre, Calgary, Alberta, Canada T2N 4N1.
anism (by producing maximal tension responses). By removing this mechanism, the apparent repriming time course is slower, because at each interval less tension can be generated in the absence of the contribution of the augmenting factor. It is not known to what extent such augmentation participates in determining tension in the adult mammalian heart, where a more complex interaction must exist between the sarcolemma and sr mechanisms.

**Introduction**

Much recent work has focused on the control of the availability of calcium, which serves to generate tension in cardiac muscle (see, for example, Chapman 1979; Fabiato 1985a, b). One of the focal points determining calcium availability stems from the fact that, after each activation, sarcolemmal calcium channels become inactivated. This entails a subsequent repriming process, which enables the channel to recover its conductance. During this repriming period, the influx of calcium in response to membrane depolarization is diminished. As repriming proceeds, the currents elicited in response to activation gradually increase.

The force-producing mechanisms must also undergo a reactivation phase (Pidgeon et al. 1980); the amount of calcium available in release stores (which is the source of calcium for activation of the myofilaments) is depleted after each contraction, owing to release from the sarcoplasmic reticulum (Chapman 1979) or from membrane binding sites (Langer 1984). There are thus two processes of reactivation (or repriming) in cardiac muscle, one involving the sarcolemmal calcium channels, and the other involving intracellular processes by which calcium is made available again for the myofilaments.

One of the key groups of physiological regulators of calcium availability in the heart is the catecholamines. Adrenaline has been shown to increase calcium influx into cardiac cells directly (Vassort et al. 1969; Reuter 1983; Bean et al. 1984; Brum et al. 1985). The repriming kinetics of calcium currents were also shown to be enhanced, both at the multicellular level (Shimoni et al. 1984) and, recently, at the single-cell level (Shimoni et al. 1987).

The present work was designed to study in detail the effects of adrenaline and isoprenaline on tension reactivation. The mechanical reactivation process is much more complex, and depends on a variety of factors. First, it must rely on the repriming of the membrane calcium influx, which triggers the contraction. Secondly, it is widely accepted that calcium is released from the sarcoplasmic reticulum (sr) network to trigger contraction, and that this release occurs at sites which are spatially distinct from sites at which the re-uptake of calcium takes place (Bassingthwaighte & Reuter 1972; Morad & Goldman 1973; Edman & Johannsson 1976; Adler et al. 1985). Thus, the repriming of the force producing apparatus probably depends on an internal translocation, or recycling of calcium.

The experiments reported here were based on two lines of evidence. On one hand, adrenaline has been shown to speed up the rate of calcium uptake into the sr (Kirchberger et al. 1972; Morad & Rolett 1972), thus ensuring a rapid relaxation (Fabiato & Fabiato 1975). If internal calcium recycling is in fact important in the repriming of tension-generating capability, catecholamines are expected to speed...
Catecholamines and tension reactivation. This has indeed been found in earlier work (Weingart et al. 1978; Fry et al. 1983). On the other hand, neonatal mammalian heart has no (or sparse) SR networks (Fabiato & Fabiato 1978; Maylie 1982). If the effects of catecholamines on repriming are mediated by internal calcium recycling through the SR, there should be a difference between the effects in adult and neonatal heart.

The major aim of this work was to attempt to assess the importance of the SR network in determining tension reactivation. This was done by comparing the effects of the catecholamines on tension repriming in several species, differing in the amount of SR in their cardiac tissue, and by comparing tension reactivation in adult and neonatal cardiac tissue.

It was hoped thereby to assess the participation of internal calcium recycling as part of the normal sequence of events in the cardiac cycle. This was thought to be of value for understanding of the control of tension production by calcium, as a function of the timing of activation. This work is of further interest in the context of recent suggestions (Fabiato 1985a, b) whereby SR calcium channels may themselves become inactivated. Tension reactivation may therefore depend on a reactivation of SR calcium channels, with no requirement for internal recycling. Preliminary results of this work have been presented earlier (Shimoni 1986).

**Methods**

The repriming of tension (also termed reactivation or restitution) has been defined and measured in several ways (see Adler et al. 1985). Here it is defined as the time-dependent process, in the course of which peak tension amplitudes recover to their steady-state level, during constant stimulation. In all the experiments the preparations were stimulated constantly at a basal rate, and extra stimuli were interposed at varying intervals, up to the basal cycle length. The degree of repriming was defined as the ratio of the amplitude of peak tension in response to the extra stimulus (labelled $T_2$), to the steady-state amplitude ($T_1$). Repriming curves were constructed by plotting these ratios against the interstimulus interval.

Atrial trabeculae were dissected from the different preparations, and pinned at one end to the bottom of the experimental chamber (1 ml volume). The other end was attached to an Akers (model 801) force displacement transducer. Silver-silver chloride stimulating electrodes were embedded in the bottom of the chamber. Stimulation was controlled by a microprocessor-controlled digital stimulator (AMI Instruments, Jerusalem). A thermistor in the bath monitored the temperature (22–25 °C for the frog experiments, and 33–36 °C for the mammalian experiments, constant to within 0.5 °C in each experiment). The solutions were heated by a Peltier thermocouple element. Flow was controlled by an LKB perfusion pump, and the solutions were bubbled with a 95% O₂–5% CO₂ mixture, to give a pH of 7.4 for the mammalian and 7.6 for the frog. The compositions of the control solutions were as shown in the table below (in millimoles per litre).

<table>
<thead>
<tr>
<th></th>
<th>NaCl</th>
<th>KCl</th>
<th>CaCl₂</th>
<th>MgCl₂</th>
<th>NaHCO₃</th>
<th>Na₂HPO₄</th>
<th>glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>frog</td>
<td>90</td>
<td>2.0</td>
<td>1.1</td>
<td>1.0</td>
<td>20</td>
<td>0.4</td>
<td>5.5</td>
</tr>
<tr>
<td>mammalian</td>
<td>140</td>
<td>5.4</td>
<td>2.0</td>
<td>1.0</td>
<td>12</td>
<td>0.4</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Tension amplitudes were recorded on a pen recorder and oscilloscope, and stored on an FM tape recorder. In cases where there was a fusion of twitches (mainly in the frog experiments) the peak amplitude of the second twitch was measured from the baseline to which the first twitch would have declined at the time of the peak of the second twitch (this was done graphically by eye).

The first set of experiments described here were done (in collaboration with A. J. Spindler) on single guinea pig ventricular myocytes. These were prepared by enzymic dispersion, by the method of Powell & Twist (1976). The cells were voltage-clamped with a single electrode, using the switching clamp method. The cells were held at $-50$ or $-45$ mV to inactivate fast sodium currents. The contractions were monitored with an optical device (Fedida et al. 1985), in which a photoelectric cell in the eyepiece of the microscope registers changes in transmitted light when a single cell contracts. A variable iris restricts the light to the single cell, which is also impaled. The current records were analysed in these experiments with a PDP 11/34 computer.

In each experiment, 2–3 (non-consecutive) trials were made for each interval. The mean repriming values for each interval were used; the average values at a given interval were taken for a group of experiments, and comparisons made between control and the different experimental conditions (by means of the t-test).

**Results**

In the first series of experiments, the temporal correlation between the calcium current and the contraction-repriming processes was examined in voltage-clamped single (guinea pig) ventricular cells. As found in multicellular preparations (Gibbons & Fozzard 1975), the membrane current reactivated much faster than contraction. It was found that adrenaline enhances both the current repriming (as reported recently by Shimoni et al. (1987)), and the contraction repriming. Figure 1 shows experimental records from a single guinea pig ventricular myocyte. For the pair of pulses given, the second of the two responses (current and contraction) is larger with adrenaline. This indicates a larger amount of reactivation which has taken place during the interval between the two pulses. The full range of intervals, showing the complete time course of reactivation, is illustrated in figure 2. This figure shows that in single cells there is a temporal disparity between current and contraction reactivation (curves $C_i$ and $C_r$ respectively). Adrenaline speeds up both processes, maintaining the temporal disparity between them (curves $A_i$ for the current and $A_r$ for contraction, in adrenaline). Because current repriming is over much before the reactivation of tension, the latter process cannot depend directly on the reactivation of the membrane influx of calcium.

The rest of the work presented here is an attempt to examine whether the effects of catecholamines on tension repriming can be attributed to an internal recycling of calcium. Earlier workers reported the enhancing effect of catecholamines on tension repriming (Figure 2 in Weingart et al. 1978; Fry et al. 1983), but this was not pursued in detail.

This work was restricted mainly to the effects of the beta-adrenergic agonist, isoprenaline, to exclude possible complications arising from $\alpha$-receptor activation.
Figure 1. Current and contraction records from single guinea pig ventricular myocytes, obtained during steady stimulation at a rate of 0.2 Hz. The membrane was held at −45 mV, and depolarized to +5 mV for 300 ms. Extra pulses were given at different intervals (300 ms in this case). (a) Voltage traces; (b) currents; (c) optically monitored contractions. The traces on the left are in control, and the ones on the right are in the presence of adrenaline (10^-8 g ml^-1). Adrenaline increases the magnitude of current (note different scales) and of contraction, and also produces more current and tension at the second pulse in the pair, indicating a larger degree of reactivation of both membrane current and tension.

Figure 2. The effects of adrenaline on the relative amount of reactivation of peak inward (calcium) current and of contraction (monitored optically), monitored simultaneously in a single ventricular myocyte (different from that shown in figure 1). The relative amount of reactivation (see Methods) is plotted for the control medium (circles) and in the presence of 10^-8 g ml^-1 adrenaline (triangles). Both current and tension reactivation are faster with adrenaline. The temporal disparity between the two processes is maintained in the presence of the drug, with current reactivation being considerably faster. (See text for details.)
(which might also affect calcium availability). Several comparisons were made, in an attempt to examine the role of the \textit{sr} network in tension repriming. The first set of experiments compared the effects of isoprenaline on tension repriming in two species, which have been reported to be different in the calcium-handling properties of their \textit{sr}. Fabiato (1982) has reported that calcium-induced calcium release from the \textit{sr} is most prominent in rat heart, and Nayler \textit{et al.} (1975) also found that microsomes from rat \textit{sr} take up more calcium than microsomes from guinea pig \textit{sr}. In these experiments, atrial trabeculae from the two species were used.

The effects of isoprenaline on tension repriming in guinea pig atria are shown in figure 3. Tension repriming was speeded up at all intervals, in all cases ($n = 11$), by low doses of isoprenaline (40 nM). The effect was completely reversible after wash-off of the drug. Higher doses (up to 4 \mu M) did not further enhance repriming.

\begin{figure}
\centering
\includegraphics{fig3}
\caption{(a) The effects of $10^{-8}$ g ml$^{-1}$ (40 nM) isoprenaline on tension repriming in the adult guinea pig atrium. Steady stimulation was given at 1 Hz, and extra stimuli were interposed at varying intervals. The ratio between the tension amplitude, in response to the extra pulse, and the steady-state tension ($T2:T1$) is plotted against the interstimulus interval in control medium (circles), with isoprenaline (triangles) and after wash-off (crosses). (b) Tension traces for three intervals before (left) and after (right) exposure to 40 nM isoprenaline. Note the larger relative amount of tension for the second pulse in each of the pairs, with isoprenaline.}
\end{figure}
In rat atria, however, the effects of isoprenaline were more complex. In 17 experiments, only 9 showed effects comparable to those found in guinea pig atria. Reactivation was enhanced in a concentration-dependent manner, with enhancement observed also at high concentrations (up to 4 μM). Figure 4 shows an example of an effect obtained with 4 μM isoprenaline, which was comparable to the one found in the guinea pig. However, in two cases the drug had very little effect, whereas in six cases there was a complex effect: at longer interpulse intervals (more than 250 ms) the repriming was enhanced by isoprenaline, as in the other cases. At shorter interstimulus intervals, however, the degree of repriming was actually less than in control. This behaviour is illustrated in figure 5.

This type of behaviour can be explained if one takes into account the major effect of isoprenaline, namely the substantial increase in the force of contraction. This is achieved by increasing the amount of calcium released from the sr network. There is therefore a balance between two effects of isoprenaline: on one hand, a greater depletion of calcium from the sr during stimulation, and on the other hand a larger re-uptake into the stores, which would speed up internal recycling. At short interstimulus intervals, an enhanced recycling could be insufficient to overcome the larger depletion of calcium from the release stores, and the degree of repriming would actually be smaller than in the control, because less calcium would be available at these intervals. Only at longer intervals, as the stores are partly refilled, does the faster recycling have an effect, with calcium being reavailable sooner than in the control. The fact that this ‘crossover’ effect was observed only
Figure 5. A second pattern of tension reactivation obtained with isoprenaline in rat atrium. In this case the time course of repriming in control (circles) intersects that obtained with 40 nM isoprenaline (triangles). Repriming is enhanced only at the longer interstimulus intervals; for the shorter intervals, less tension is produced with the drug (see text). This behaviour was seen in six other cases.

in the rat is compatible with the results of Fabiato (1982) and Nayler et al. (1975), who found a larger release and uptake of calcium in the rat SR.

A different approach, to try and establish whether internal calcium recycling occurs and whether it is affected by isoprenaline, was to compare the effects of the drug in an adult and a neonatal atrial preparation, which contains very little SR (Maylie 1982). Tension in neonatal heart must therefore depend directly on activation by calcium influx. Indeed, no calcium-induced calcium release was detected in neonatal heart (Fabiato & Fabiato 1978; Fabiato 1982).

In a series of experiments on neonatal guinea pig and rat atria, the basal tension-repriming kinetics were found to be faster than in the adult, as shown earlier in the cat atrium by Maylie (1982). The effects of isoprenaline on tension itself were similar to those in adult heart: the drug produced a dose-dependent positive inotropic effect. However, the effects on tension repriming were quite different from the results presented above. In neonatal guinea pig atria (n = 9), for a wide range of concentrations (40 nM to 4 μM), isoprenaline did not affect tension repriming. An example is shown in figure 6.

The results in the neonatal rat were again more complex. Not only was tension repriming not enhanced, but isoprenaline actually retarded the process. Although the mean repriming values (n = 19) with the drug were not significantly slower (p ≈ 10%), in individual cases the retardation could be quite striking. An example is shown in figure 7. The mean repriming values for the different intervals in neonatal guinea pig and rat atria are shown in tables 1 and 2.
Figure 6. The effects of isoprenaline on tension repriming in neonatal guinea pig atrium. The relative repriming values, plotted as $T_2/T_1$ values against the interval, were unaffected by either low (40 nM) or high doses (4 μM) of isoprenaline, plotted as triangles and crosses, respectively. The values in the control medium are shown as circles.

Figure 7. The effect of isoprenaline on tension repriming in neonatal rat atrium. The time course of reactivation in the control medium is much faster than in the adult (compare with figure 4). In this case both a low dose of 40 nM (triangles) and a high dose of 4 μM (crosses) of isoprenaline slowed down the reactivation process. The inset shows tension traces for one interval, showing that isoprenaline gives a large inotropic effect in the neonate, but a considerably smaller degree of reactivation for this interstimulus interval. In other cases reactivation was also slowed down by isoprenaline, usually to a smaller extent.
Table 1. Effect of isoprenaline on repriming \((T2/T1)\) in the neonatal guinea pig atrium

(Values are given as means ± standard deviations for each interval.)

<table>
<thead>
<tr>
<th>interval/ms</th>
<th>control</th>
<th>isoprenaline ((10^{-8} \text{ g ml}^{-1}))</th>
<th>isoprenaline ((10^{-6} \text{ g ml}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>0.367 (0.233)</td>
<td>0.376 (0.292)</td>
<td>0.402 (0.285)</td>
</tr>
<tr>
<td>350</td>
<td>0.411 (0.193)</td>
<td>0.412 (0.269)</td>
<td>0.456 (0.252)</td>
</tr>
<tr>
<td>400</td>
<td>0.466 (0.170)</td>
<td>0.431 (0.245)</td>
<td>0.486 (0.240)</td>
</tr>
<tr>
<td>450</td>
<td>0.512 (0.163)</td>
<td>0.488 (0.225)</td>
<td>0.557 (0.213)</td>
</tr>
<tr>
<td>500</td>
<td>0.567 (0.147)</td>
<td>0.542 (0.208)</td>
<td>0.615 (0.196)</td>
</tr>
<tr>
<td>550</td>
<td>0.624 (0.129)</td>
<td>0.496 (0.102)</td>
<td>0.593 (0.139)</td>
</tr>
<tr>
<td>600</td>
<td>0.691 (0.112)</td>
<td>0.631 (0.177)</td>
<td>0.709 (0.143)</td>
</tr>
<tr>
<td>700</td>
<td>0.766 (0.085)</td>
<td>0.732 (0.151)</td>
<td>0.793 (0.114)</td>
</tr>
<tr>
<td>800</td>
<td>0.856 (0.067)</td>
<td>0.821 (0.115)</td>
<td>0.873 (0.083)</td>
</tr>
<tr>
<td>900</td>
<td>0.900 (0.041)</td>
<td>0.882 (0.029)</td>
<td>0.917 (0.043)</td>
</tr>
</tbody>
</table>

Table 2. Effect of isoprenaline on repriming \((T2/T1)\) in neonatal rat atrium

(Values are given as means ± standard deviations for each interval.)

<table>
<thead>
<tr>
<th>interval/ms</th>
<th>control</th>
<th>isoprenaline ((10^{-8} \text{ g ml}^{-1}))</th>
<th>isoprenaline ((10^{-6} \text{ g ml}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.492 (0.095)</td>
<td>0.391 (0.103)</td>
<td>0.418 (0.178)</td>
</tr>
<tr>
<td>125</td>
<td>0.619 (0.117)</td>
<td>0.488 (0.184)</td>
<td>0.580 (0.140)</td>
</tr>
<tr>
<td>150</td>
<td>0.641 (0.166)</td>
<td>0.595 (0.117)</td>
<td>0.567 (0.194)</td>
</tr>
<tr>
<td>175</td>
<td>0.687 (0.228)</td>
<td>0.630 (0.260)</td>
<td>0.770 (0.118)</td>
</tr>
<tr>
<td>200</td>
<td>0.741 (0.161)</td>
<td>0.607 (0.260)</td>
<td>0.672 (0.240)</td>
</tr>
<tr>
<td>250</td>
<td>0.817 (0.117)</td>
<td>0.688 (0.246)</td>
<td>0.766 (0.220)</td>
</tr>
<tr>
<td>300</td>
<td>0.883 (0.080)</td>
<td>0.758 (0.182)</td>
<td>0.819 (0.170)</td>
</tr>
<tr>
<td>350</td>
<td>0.877 (0.069)</td>
<td>0.737 (0.168)</td>
<td>0.810 (0.194)</td>
</tr>
<tr>
<td>400</td>
<td>0.908 (0.067)</td>
<td>0.877 (0.070)</td>
<td>0.899 (0.075)</td>
</tr>
<tr>
<td>500</td>
<td>0.931 (0.055)</td>
<td>0.917 (0.049)</td>
<td>0.951 (0.025)</td>
</tr>
<tr>
<td>600</td>
<td>0.931 (0.047)</td>
<td>0.932 (0.034)</td>
<td>0.955 (0.030)</td>
</tr>
<tr>
<td>700</td>
<td>0.945 (0.037)</td>
<td>0.957 (0.020)</td>
<td>0.960 (0.028)</td>
</tr>
</tbody>
</table>

Thus, the lack of an \(sr\) network seems to prevent the enhancing effect of isoprenaline on tension reactivation. The retardation of repriming in the rat may be the result of an effect of isoprenaline on a further underlying process, which temporally overlaps the repriming process. Such an additional process will be discussed below.

The final set of experiments looked into the effects of isoprenaline on tension reactivation in the frog atrium, which also possesses little \(sr\) (see, for example, Page & Niedergerke 1972) and shows no calcium-induced calcium release from internal stores (Fabiato & Fabiato 1978). It is well established that tension in frog heart is directly activated by calcium current (Niedergerke et al. 1976; Morad et al. 1983). Furthermore, a very close correlation has been found between the effects of isoprenaline on calcium current and its effects on tension in frog atrium (Ouedraogo et al. 1982). It was therefore of interest to compare the effects of isoprenaline on tension reactivation in frog with those in neonatal mammalian heart.
The effects of isoprenaline on tension repriming in frog atrium turned out to be rather complex. An initial standard concentration of $10^{-7}$ g ml$^{-1}$ (0.4 $\mu$m) could affect repriming in opposite ways in different preparations, either enhancing the process (in 8 cases) or retarding it (in 8 other cases). This puzzling feature was resolved by using a wider concentration range.

It turned out that low concentrations of isoprenaline (usually $10^{-8}$ g ml$^{-1}$ (40 nM)) speeded up tension reactivation, whereas high concentrations (4 $\mu$m) retarded the process. At intermediate concentrations, such as had been used initially, either effect could be obtained in different preparations. This presumably means that different levels of drug are required to shift an enhancing to a retarding effect in different preparations. In some cases, an intermediate concentration produced a 'crossover' effect: enhanced repriming at short interstimulus intervals, and less repriming at the longer intervals (this 'crossover' effect is the opposite of the one shown above in the adult rat atrium). An example is shown in figure 8a. In this case the lower concentration ($10^{-8}$ g ml$^{-1}$) enhances repriming at all intervals, whereas a higher concentration shifts the repriming curve so that it

![Figure 8](http://rspb.royalsocietypublishing.org/)

**Figure 8.** The effects of isoprenaline on tension repriming in the frog atrium. (a) $T2/T1$ values at different intervals in control (circles) and with two concentrations of isoprenaline: $10^{-8}$ g ml$^{-1}$ (40 nM), shown as triangles, enhanced repriming at all intervals. A concentration of $5 \times 10^{-7}$ g ml$^{-1}$ (crosses) shifts the time course of repriming, so that it intersects with the control curve. There is now more reactivation at short intervals, and less at long intervals. (b) Tension traces obtained at one interval (different preparation): the top traces are in control, the middle traces with $10^{-8}$ g ml$^{-1}$ and the bottom traces with $2 \times 10^{-7}$ g ml$^{-1}$ isoprenaline. The lower concentration gives more reactivation, and the higher concentration less reactivation, for this interval. (See text for possible interpretations.)
intersects the control curve. Figure 86 shows that for a given interval, the relative amount of repriming can be either enhanced or reduced, depending on the amount of drug in the medium.

The interpretation of this effect is based on the assumption that after each activation, there are probably two overlapping time-dependent processes which can determine the magnitude of tension produced. This was first proposed by Braveny & Kruta (1958) and received experimental support in the findings of Noble & Shimoni (1981). The latter described a positive 'staircase' effect, whereby stimulation of frog atrial trabeculae within a certain interval range gives rise to augmented calcium influx. Although the nature of this augmenting mechanism is elusive, it is often manifest as a non-monotonic time course of calcium current repriming (figures 9 and 10 in Shimoni 1981). In such cases, for certain interpulse intervals, the second of two pulses elicits more current than the steady-state level (giving repriming values larger than 1.0). The augmenting mechanism then subsides; for longer intervals repriming values return towards 1.0. Tension reactivation in frog atrium also often shows similar behaviour, with an intermediate interval range that exhibits augmented responses (see below).

By assuming a superposition of an augmentation mechanism onto the basal repriming process, one can reconstruct a theoretical time course for tension reactivation. This can be done by (arbitrarily) assigning values for the time constant of the basic repriming process, the time constant for decay of the augmentation process, and an initial amount of augmentation elicited by the preceding activation. For different times after each activation, the amount of tension produced will be the sum (algebraic for simplicity) of the contributions of the two processes. Plotting the 'net' amount of tension for different 'interstimulus' intervals (different times after a preceding activation) will produce a time course of repriming of tension, such as can be measured experimentally. This may be termed the apparent repriming time course. The actual shape of the reconstructed time course of repriming will vary according to the relative contribution of the augmentation process.

By following such a procedure, either monotonic or non-monotonic time courses could be reconstructed, which closely resemble the ones measured experimentally. Figure 9a illustrates an example of such a theoretical reconstruction, leading to a time course of apparent repriming showing an 'overshoot'; the figure also illustrates how the actual measured tension can be derived from the addition of two underlying contributions at varying times.

The complex effects of isoprenaline can then be explained by assuming differential effects on the two overlapping processes. The lower concentration of isoprenaline probably speeds up the basal repriming process, as suggested by experiments that directly monitored calcium currents (Shimoni et al. 1984). The high concentrations, by giving maximal current and tension responses, eliminate the augmentation process, since at some point the response would reach a maximal level and no augmentation would then be possible. If the apparent repriming process under control conditions is a reflection of two superimposed process (figure 9a), the removal of the contribution of an augmenting mechanism will produce
Catecholamines and tension reactivation 243

243

Figure 9. Simulation of tension repriming in frog atrium. (a) Relative tension plotted against interstimulus interval. The actual amount of tension produced (curve denoted by arrow) is assumed to consist of the combined contributions of two processes: a basal repriming process, which grows monoexponentially towards the steady-state level (100%). Superimposed on this is an augmentation process. This starts at some arbitrarily chosen value (greater than 100%) and decays monoexponentially towards the steady-state amplitude. The actual amount of tension produced at any interval is the sum of the two contributing processes. Plotting the net amount of tension for different intervals gives the apparent repriming time course. By choosing different values for the two time constants and for the initial amount of augmentation, different patterns can be obtained, either monotonically increasing tension, or, as here, a time course showing an overshoot. In this particular case, the time constants of repriming and decay of augmentation were 300 and 800 ms, respectively, and the initial augmentation was 150%. (b) The two time courses can be simulated to intersect by changing two parameters simultaneously. The repriming time constant was 250 ms in both cases. In the curve marked by circles, the initial augmentation was 120%, decaying with a time constant of 600 ms; in the curve marked by triangles, the initial augmentation was 60%, and decayed with a time constant of 200 ms. This may represent the effects of intermediate concentrations of isoprenaline.

a slowing down of the apparent repriming time course (measured experimentally) relative to the control situation.

At the intermediate range a crossover effect can be obtained by assuming a dual action of isoprenaline. For example, isoprenaline at this concentration may induce a larger initial amount of augmentation. Shimoni et al. (1984) found larger
positive-current staircase effects with isoprenaline; their results would support this assumption. If it is assumed that isoprenaline also causes a faster decay of augmentation, e.g. by a faster removal of calcium, it is possible to obtain a repriming time course that intersects with the 'control' curve. An example is shown in figure 9b. Other combinations in which two parameters are changed could also give intersecting time courses of repriming.

The final set of experiments tested some of these assumptions. If there are indeed two mechanisms that overlap to produce tension at short intervals, it was assumed that the contribution of the augmenting mechanism could be removed by other means, in addition to high levels of isoprenaline. It was assumed that elevating internal calcium levels might also saturate this mechanism, because many tension augmenting phenomena, such as post-extrasystolic potentiation, are abolished by high levels of calcium (Koch-Weser & Blinks 1963). On the abovementioned hypothesis, this would leave only the basal repriming process. The net effect would be a slowing of the apparent repriming time course in relation to control.

Two methods for elevating calcium were used. Raising external calcium levels leads to a rise in intracellular calcium (Sheu & Fozzard 1982). A doubling of external calcium was found to slow down the repriming of tension. This is shown in figure 10, which also shows the progression of the change with time, as internal calcium is presumably rising. Note that in the control repriming curve there is a marked overshoot (obvious also in the larger tension amplitude in the second of the two tension traces in control, figure 10b). This augmented response is removed by the higher calcium level.

![Figure 10](http://rspb.royalsocietypublishing.org/Downloaded from 244 Y. Shimoni)

**Figure 10.** The effects of increasing extracellular calcium on tension repriming in the frog atrium. (a) $T_1/T_2$ values at different intervals in control Ringer, containing 1.1 mM (circles) and 2.2 mM calcium (triangles). Note that there is an overshoot in the repriming time course in 1.1 mM, which is abolished in 2.2 mM calcium (in addition to the considerable retardation of repriming at the higher calcium level). (b) Tension traces for a given interval (basal stimulation rate of 0.5 Hz). The top trace is in 1.1 mM calcium (note slight augmentation of second twitch). Below are traces obtained for the same interval, at different times (in minutes) after changing to 2.2 mM calcium. The $T_2/T_1$ ratio gradually decreases with time, presumably as intracellular calcium rises.
Finally, it was of great interest to examine the effect of the α-adrenergic agonist phenylephrine. α-Receptor activation was found previously to have positive inotropic effects in frog atrium by Niedergerke & Page (1981); they observed a positive inotropic effect in some of the preparations, whereas in others there was no effect. This positive inotropic effect is now thought to be due to release of internal calcium by activation of the phosphatidylinositol pathway, with inositol triphosphate as the second messenger (see, for example, Homey & Graham 1985). In a series of experiments with phenylephrine, the effects on reactivation were examined in those preparations in which there was also a positive inotropic effect. Phenylephrine (1 μM) was also found to retard the tension reactivation process. This is illustrated in figure 11. Again, the elevated calcium may saturate the augmenting process and remove its contribution. This will be apparent as a slower reactivation, because at short intervals less tension is generated, in the absence of the additional augmenting process.

**Discussion**

The results presented here illustrate two major aspects of tension generation in cardiac muscle. The different effects of isoprenaline on tension reactivation in the adult guinea pig and the adult rat, together with the lack of enhancing effect in the neonatal atria, all strongly support the idea that calcium is translocated between different sites in cardiac cells. The time course of this translocation is a major contributing factor in determining tension reactivation in adult mammalian heart. The amount of tension produced at short intervals after each activation will depend on a balance between the amount of calcium released from the stores and the speed at which they are refilled. Isoprenaline affects both processes, leading to a complex effect in the rat atrium, with repriming time courses that intersect.
with the control curves. The fact that the complex behaviour is seen only in the rat is consistent with the observations that the rat SR takes up and releases more calcium than the guinea pig SR network.

It is also clear that tension repriming does not directly depend on the repriming of sarcolemmal calcium channels (although obviously with no calcium influx there is no contraction). The reactivation of calcium channels in the SR network may also contribute to tension repriming, but this is difficult to test experimentally.

The striking lack of effect of isoprenaline on tension repriming in the neonatal guinea pig is probably due to a lack of SR in the neonate (Maylie 1982). Other features of cardiac performance which are thought to depend on the SR, such as staircase effects, are also different in the neonate, and change with development (Lee & Downing 1978; Langer et al. 1975). Tension in the neonatal heart is presumably activated directly by membrane calcium currents. This is probably the reason for the faster tension repriming in the neonate, as compared with the adult, since the membrane current reprimes much more rapidly. In the neonatal tissue, tension reactivation is therefore different from that in the adult tissue, not only in its kinetics (Maylie 1982) but also in the response to isoprenaline. This again emphasizes the central role of the SR in determining the reactivation process in adult mammalian heart.

The repriming of membrane calcium channels was found to be faster in the presence of isoprenaline, in adult mammalian ventricular cells (Shimoni et al. 1987). If tension reactivation in the neonate depends only on calcium-channel repriming, the lack of effect of isoprenaline in neonatal cells seems puzzling. This may be due to the fact that the process is already very rapid, or to a different response of the membrane calcium repriming process to isoprenaline. This again underlies the retardation of repriming in the presence of isoprenaline in the neonatal rat atrium, is that as in the frog, there is some calcium-current augmenting mechanism, which overlaps the repriming process. There is much evidence for this. Positive-current staircase effects have been described in mammalian tissue as well as in frog (Payet et al. 1981; Brown et al. 1984; Fedida et al. 1985). Furthermore, in determining calcium current repriming in single cells, an overshoot is often evident (Shimoni et al. 1987). Isoprenaline suppresses this augmenting factor, as well as enhancing repriming (figure 6 in Shimoni et al. 1987). If in the neonatal rat the major effect of isoprenaline is to suppress an augmenting contribution, the result will be a slower repriming. In the neonatal guinea pig, the opposing effects of isoprenaline (enhanced repriming and reduced augmentation) may cancel out, giving no net effect.

In summary, the experimental results bear out the original hypothesis, that in cardiac tissues differing in, or lacking, an SR network there are different effects of isoprenaline on tension repriming. The results are compatible with the idea that, in the presence of such a network, calcium recycling within the cell plays a major role in determining the amount of tension produced at early intervals. The experiments in the different tissues point to an additional underlying effect, which in certain conditions leads to augmented responses.

Tension-augmenting phenomena have long been recognized and studied (Koch-
Weser & Blinks 1963). However, the augmentation referred to here is distinct from tension potentiation that occurs as a result of different degrees of loading of the SR, such as post-extrasystolic potentiation. The augmenting process, which affects repriming, probably results from processes occurring at the sarcolemma. Previous workers also provided evidence for changes in calcium currents produced by calcium itself (Isenberg 1977; Marban & Tsien 1982), in addition to the positive staircase effects of calcium currents, mentioned above.

This augmenting process is relevant in those cases where tension reactivation depends on calcium current reactivation, namely, when there is little SR present. Previous results showed that the augmenting mechanism overlaps the membrane calcium current reactivation (Shimoni 1981; Shimoni et al. 1987). The present results show that this mechanism also coincides with tension reactivation, and can explain the complex effects of isoprenaline in the frog atrium, and perhaps in the neonatal tissue as well. Although the nature of this augmenting mechanism remains elusive, it acts at short intervals after each activation, and by interacting with the basal process of repriming ultimately determines how much tension is produced.

Interestingly, several cardiac drugs have also been shown to produce tension repriming curves which intersect with control curves (Wasserstrom & Ferrier 1982). Further analysis may show a differential action of cardiac drugs on these two underlying processes, which determine tension magnitude.

This work was supported by the Israel Academy for Sciences and Humanities and by the Myra Kurland Fund. Y.S. thanks the Revson Foundation for their support.

References


Brum, G., Osterrieder, W. & Trautwein, W. 1984 Beta-adrenergic increase in the calcium conductance of cardiac myocytes studied with the patch clamp. Pfliigers Arch. Eur. J. Physiol. 401, 111–118.


Fedida, D., Noble, D., Shimoni, Y. & Spindler, A. J. 1985a Inward current and contraction in mammalian cardiac cells: interventions that augment or reduce current staircase. *J. Physiol., Lond.* 366, 84P.


