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Control of interval-force relation in canine ventricular myocardium studied with ryanodine

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1 The mechanism of post-extrasystolic, rest and frequency potentiation was studied in canine isolated ventricular muscle.

2 Ryanodine, which impairs Ca availability from the sarcoplasmic reticulum (SR), reduced the amplitude of the extrasystole less than that of the steady state contraction. Ryanodine also inhibited post-extrasystolic potentiation and converted rest-potentiation into rest depression. Rest-potentiation was blocked preferentially by ryanodine compared to post-extrasystolic potentiation. An increase in the contribution of extracellular Ca to the extrasystolic contraction could not entirely account for the post-extrasystolic potentiation.

3 Prolonged rest, by itself, also caused depression of the first post-rest contraction. During rest-potentiation, SR Ca seemed to play a greater role in contraction than transmembrane Ca influx. However, the ability of the 'release pool' of Ca in the SR to be reprimed after a contraction was reduced. This was seen as a decrease in post-extrasystolic potentiation elicited immediately after rest.

4 A decrease in stimulus interval was associated with a transient decrease in contraction amplitude followed by an increase. An abrupt increase in stimulus interval had the opposite effect. Ryanodine blocked the initial transient changes and accelerated the delayed changes. These results suggest that the transient changes in contraction after sudden changes in drive interval are dependent on the SR.

5 Transmembrane Ca entry and the rate of recovery of the Ca release process (repriming) in the SR after a contraction seem to be interval-dependent. The data also indicate that different mechanisms are involved in post-extrasystolic and rest-potentiation.

6 The results are consistent with a model which proposes 'recirculation' of activator Ca within the SR after a contraction or of the presence of an appreciable amount of inactivation of the SR Ca release process during normal stimulation. An increased pool of releasable Ca due to longer recirculation time or a time-dependent decay in the level of inactivation of Ca release from the SR may give rise to rest-potentiation.

Introduction

Mammalian ventricular myocardium shows complex changes in contractility when the rate or pattern of stimulation is changed. These are seen in the form of frequency potentiation (FP), post-extrasystolic potentiation (PESP) and rest-potentiation (RP). The latter is seen in ventricular preparations from some mammalian species (e.g. cat and dog; Koch-Weser & Blinks, 1963; Endoh & Iijima, 1981) but not in others (rabbit; Edman & Johannsson, 1976). A model of excitation-contraction coupling which would account for the interval-force relation proposes the recycling of activator Ca from an uptake site to a release site in the sarcoplasmic reticulum (SR), during the interval between contractions (e.g. Wood *et al.*, 1969; Morad & Goldman, 1973; Allen *et al.*, 1976; Edman & Johannsson, 1976, Wohlfart & Noble, 1982; Schouten *et al.*, 1987). The size of the releasable pool of Ca, at the moment of onset of contraction, is thought to be a function of the time elapsed after the last release of Ca and the degree of refilling of this pool, due to Ca influx during the preceding beat and by translocation within the SR

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(Wohlfart & Noble, 1982). Ca influx across the sarcolemma (SL) during an action potential may contribute directly to contraction but the magnitude of this is uncertain and may be variable (New & Trautwein, 1972). It is quite possible that the contributions may vary with different physiological situations. The present study examined the relative contributions of the SR and extracellular divalent cation to contraction during short-term alteration of contractility due to the interval-force relation. Ryanodine, an alkaloid which impairs Ca release from the SR (Sutko *et al.*, 1979), was used as a tool to provide some insight into the cation pools involved in the interval-force relation.

Methods

Preparation

Hearts were removed from mongrel dogs (3–10 kg) of either sex, under pentobarbitone anaesthesia (30 mg kg^{-1} i.p.). These were immersed in cold, oxygenated Krebs-Henseleit (KH) solution. Thin, free-running trabeculae (< 1.5 mm) were tied with 6.0 silk thread at each end and removed from the wall of the ventricle. Tissues were usually obtained from the right ventricle where thin trabeculae are more abundant.

Recording techniques

The tissue was tied to the base of a stimulating electrode and placed in a 20 ml vertical tissue bath containing KH solution bubbled with 95% O_2 and 5% CO_2. The temperature of the bath was maintained at 37.0 ± 0.2°C. The free end of the tissue was connected with a stainless steel wire to a isometric force-displacement transducer (Grass FT-O3C). A stimulator (F. Haer Instruments; Pulsar 6i) connected to a custom built computer-controlled programmable pulse generator (Boyechko & Bose, 1984) or to a programmable stimulus parameter incrementor (F. Haer Instruments; Pulsar ICR) provided square wave stimuli of 3 ms duration to punctate platinum electrodes. Stimulus amplitude was adjusted to about 10–20% above threshold. The resting tension was increased until the maximum twitch tension at a basic stimulus interval (basic cycle length) of 2000 ms was obtained and then the tissues were equilibrated for 1 h. Contractions and stimuli were stored on a Hewlett-Packard model 3960 instrumentation tape recorder and were simultaneously displayed on a waveform analyser (Data Precision; Model Data 6000) and a 4 channel chart recorder (Grass Model 7; Gould Brush; Model 440) and photographed on Polaroid film or plotted on a digital plotter (Hewlett Packard; Model 7475B).

Stimulation protocol

A basic cycle length of 2000 ms was chosen for most experiments. A train of 10 or more stimuli was followed after a variable interval by another similar train. The first pulse of the second train was considered the test pulse. The stimulator was programmed to increase the interval between the 2 trains by a predetermined amount after each train. This varied between 200 ms to 1200 s. A beat following a coupling interval of less than the basic cycle length was referred to as an extrasystole and that longer than the basic cycle length was referred to as a post-rest beat. The interval between the extrasystole and the subsequent pulse (post-extrasystole) was kept the same as the basic cycle length. A sufficient number of conditioning pulses necessary to ensure complete recovery after rest were inserted before the test sequence.

Statistical analysis of data

Each experiment consisted of at least 5 trials, unless specifically mentioned. In self-controlled experiments with single treatment, statistical significance was measured by use of Student's paired t test. When the experiment involved more than 2 groups, One Way Analysis of Variance in conjunction with Duncan's New Multiple Range Test (Steel & Torrie, 1960) was employed. A P value of < 0.05 was considered statistically significant.

Solutions and drugs

Krebs-Henseleit solution (KH) had the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.4, Na_2HPO_4 1.7, $NaHCO_3$ 25, glucose 11.1. Ryanodine was a gift from Dr R. Rogers (Merck Sharp and Dohme) and was dissolved in 0.1 N HCl.

Results

Effect of changes in stimulation frequency

In KH solution, a decrease in the basic cycle length, in the range of 200 to 600 ms, resulted in increased tension (Figure 1 a1, b1, c1 and d1). Tension of the first contraction at the shorter basic cycle length was smaller and then gradually increased to a new and higher steady state.

An increase in the coupling interval between beats led to an increase in the tension of the first contrac-

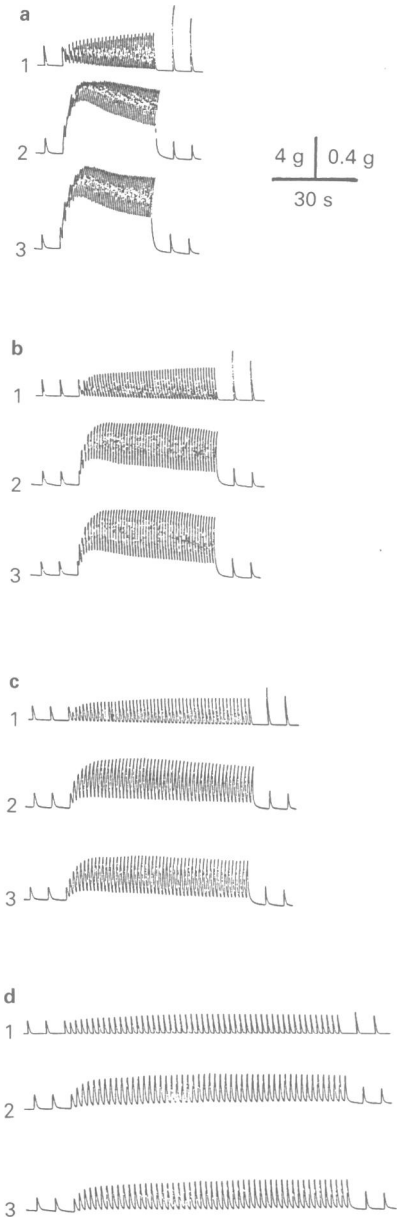

Figure 1 Effect of reduction of the basic cycle length (BCL) from 2000 ms to (a) 200, (b) 300, (c) 400 and (d) 600 ms followed by resumption of normal BCL. The first two beats in each group are at the normal (longer) driving interval. Panel 1 is the response in the untreated tissue. The potentiation of the first beat on resuming the normal coupling interval is inversely proportional to the change in interval. Panels 2 and 3 are 30 min and 60 min after 10^{-7} M ryanodine. Note higher recording gain. Positive staircase is maintained but potentiation due to change from a short to a long basic cycle length is markedly reduced.

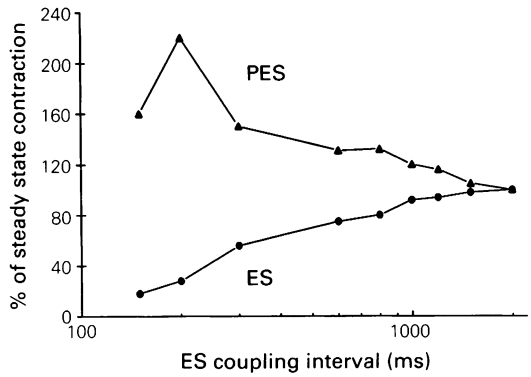

Figure 2 Effect of varying the coupling interval on extrasystolic (ES) and post-extrasystolic (PES) contractions.

tion at the longer interval. The amplitude of the first contraction at the longer coupling interval was inversely proportional to the immediately preceding coupling interval. The subsequent contractions (not shown in the record) developed decreasing amounts of tension until a new steady state was reached after about 10 contractions. This behaviour resembles rest-potentiation, to be described later.

Post-extrasystolic potentiation

A single premature extrasystole caused the potentiation of the next normal beat (post-extrasystolic potentiation). The extrasystolic contraction was smaller than the steady state contraction at all test pulse intervals between 150–800 ms (Figures 2 and 3).

The post-extrasystolic contraction was potentiated when the extrasystolic interval was less than 1000 ms. The magnitude of potentiation increased with shortening of the test pulse interval to 200 ms, but decreased again at a coupling interval of 150 ms.

Rest-potentiation

The first contraction after an interruption of stimulation produced more tension than steady state contractions (Figure 3). This will be referred to as 'rest-potentiation'. The effects of interruptions lasting between 5 s to 1200 s were studied at a basic cycle length of 2000 ms. After a pause longer than 240–1200 s the post-rest beat was usually depressed (post-rest depression). This effect was seen at shorter rest intervals in the young dogs (<6 months old) but at longer intervals in adult dogs. Maximum potentiation of the post-rest beat usually occurred after a rest period of 60–120 s (longer period applies to older

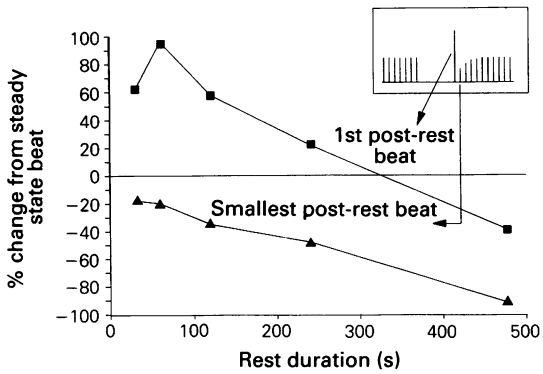

Figure 3 Effects of varying rest intervals on post-rest contractions. The basic cycle length was 2000 ms. (■) Represent the first beat after rest while (▲) denote the smallest beat after rest (tension undershoot). This has been shown in the inset. The figure shows that post-rest undershoot in tension is related to rest duration.

dogs). Potentiation of the initial few beats following rest, was followed by a decrease in amplitude below the pre-rest level and a final gradual return to the control level. The magnitude of this tension undershoot was proportional to the rest duration (Figure 3).

Effects of ryanodine

The SR has been postulated to play an important role in interval-related changes in cardiac contractility (Johnson & Kuohung, 1968; Wood *et al.*, 1969; Manring & Hollander, 1971; Bassingthwaighte & Reuter, 1972; Morad & Goldman, 1973; Forrester & Mainwood, 1974; Kaufmann *et al.*, 1974; Allen *et al.*, 1976; Chapman & Leoty, 1976). Since ryanodine has been postulated to impair the release of Ca (Sutko *et al.*, 1979), increase its leakage from the SR (Hilgeman, 1982; Hunter *et al.*, 1983) and also cause structural changes in the vicinity of the t-tubule and lateral cisternae (Penefsky, 1974), its effect on the various potentiation phenomena was examined. Steady state contractions, obtained in the presence of normal Ca (2.5 mM) at a basic cycle length of 2000 ms, were reduced to about 5–10% of control in the presence of ryanodine (10^{-7} M–10^{-5} M). The reduction in contraction amplitude was irreversible and time-dependent.

Frequency potentiation In the presence of ryanodine (10^{-7} M) an abrupt change in basic cycle length from 2000 ms to 200, 300, 400 or 600 ms resulted in a progressive increase in the peak level of contractions without the initial decrease seen in normal tissues (Figure 1, panels 2 of a, b, c and d). The increase in contraction amplitude occurred in two phases, an initial fast one followed by a smaller slow increase. The opposite was seen when the interval was abruptly increased. The initial rapid phase of increase in contraction amplitude on shortening the basic cycle length occurred faster in the presence of ryanodine than in the untreated tissue (Figure 4).

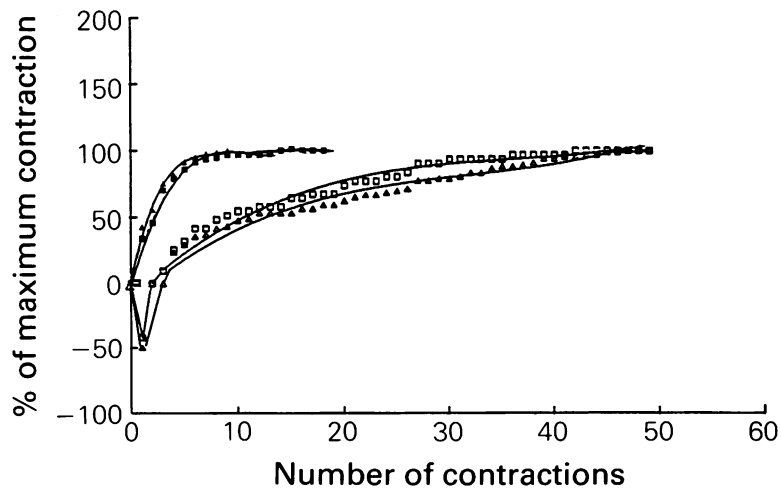

Figure 4 Effect of a decrease in the basic cycle length from 2000 ms to 300 ms (□, ■) or 200 ms (△, ▲) in control (□, △) and ryanodine (10^{-7} M)-treated trabecula (■, ▲). Note that in contrast to the control preparations (also Figure 1 a1, b1 and c1) there is an absence of the transient negative inotropic effect of the shorter coupling interval and also a faster rate of attaining peak amplitude in the ryanodine-treated tissue. The data are from 4 experiments. All experimental points in the ryanodine-treated tissue except the first one are significantly different ($P < 0.05$ ANOVA/Duncan's multiple range test) from the corresponding ones in the control tissue.

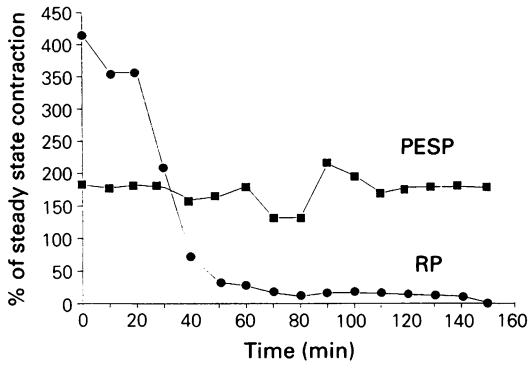

Figure 5 Effect of different durations of treatment with ryanodine (3×10^{-8} M) on post-extrasystolic potentiation (PESP, after a 300 ms extrasystole) and rest-potentiation (RP, after 120 s rest). Basic cycle length of normal conditioning beats was 2000 ms. Note the preferential depression of the post-rest beat.

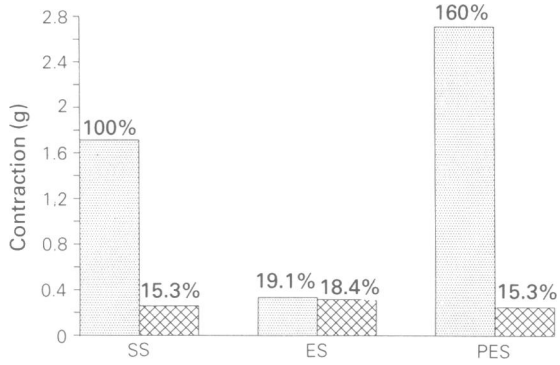

Figure 6 Contribution of Ca from the SR and extracellular space to normal (SS), extrasystolic (ES) and post-extrasystolic (PES) contractions, in the presence of normal extracellular Ca, in control (stippled columns) and ryanodine-treated (hatched columns) tissues. The basic cycle length was 2000 ms and extrasystolic coupling interval was 300 ms. Numbers above the columns refer to contraction amplitude expressed as a % of steady state contraction in the normal muscle. For explanation see Results and Discussion.

Ryanodine also markedly reduced the potentiation seen immediately after returning to the initial basic cycle length (Figure 1, panel 2 of a, b, c and d). The effects of ryanodine increased over a period of time (i.e., 30 vs 60 min; Figure 1, panels 2 vs 3).

Post-extrasystolic potentiation Unlike control preparations, in the presence of ryanodine, extrasystolic beats were larger than the steady state beats. The size of this extrasystolic contraction increased at shorter coupling intervals. This was in contrast to the behaviour of the extrasystole in the untreated muscle. SR plays a relatively insignificant role in the presence of ryanodine (Sutko *et al.*, 1979). Hence contractions after treatment with ryanodine are mainly dependent on extracellular Ca. The post-extrasystolic potentiation was not diminished in the presence of 3×10^{-8} M ryanodine (Figure 5) and was only partially blocked with a concentration of 10^{-7} M. A larger concentration of the alkaloid (10^{-6} M–10^{-5} M) completely abolished post-extrasystolic potentiation.

It has been proposed that activator Ca entering during the extrasystole may recirculate back to the release site in the SR and cause potentiation of the post-extrasystolic beat. To examine this 5 trials of the type shown in Figure 6 were performed.

In this experiment the basic cycle length was 2000 ms and the extrasystolic interval was 300 ms. Ryanodine blocked the steady-state contractions to 15.3% of that in the normal muscle. Hence Ca from the SR supported roughly 84.7% of the contraction in the normal muscle. Extrasystoles were 19.1% of the steady state beats in the control muscle. These were decreased after ryanodine (10^{-6} M) to only 18.4% of the control SS beats without ryanodine. In other words the post-ryanodine extrasystoles were 3.7% smaller than those before adding the drug. This difference was statistically not significant; $P > 0.05$ ($n = 4$). Hence the SR appeared to play an insignificant role in the extrasystolic contraction. In the presumed absence of contribution of Ca from the SR in the ryanodine-treated tissue, the extrasystoles were about 25% larger than the steady state beats in the ryanodine-treated tissue. When expressed as a % of the steady state amplitude of the contraction in the absence of ryanodine, this difference was 3.1%. It is not clear whether this increase in the size of the extrasystole in the ryanodine-treated muscle is entirely due to increased extracellular Ca influx or due to reduced buffering of Ca_i by the SR. However, this is an estimation of the maximum increase in contraction possible through enhanced transmembrane Ca entry. It seems unlikely that this small increase in Ca influx or increased intracellular availability can fully account for the observed post-extrasystolic potentiation of 60%.

Rest-potentiation Ryanodine (10^{-6} M) completely abolished rest-potentiation at all intervals between 5 s–1200 s. The characteristic response in the presence of ryanodine was a reduction in the size of the first post-rest beat to below the level of the preceding steady-state contraction followed by a gradual recovery (Figure 7 inset).

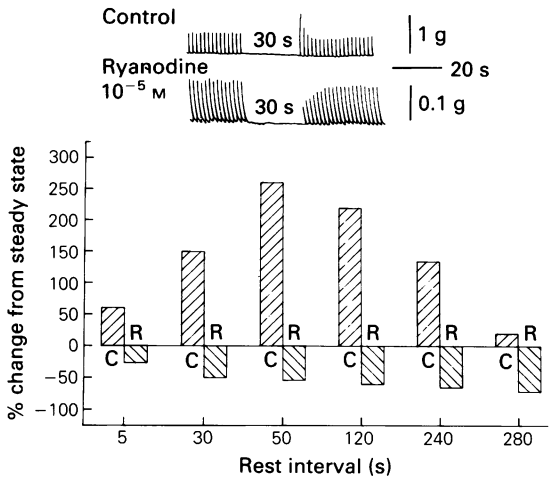

Figure 7 Comparison of the effect of rest in the normal muscle (C) and ryanodine (10^{-6} M, 45 min)-treated muscle (R). Inset shows that the post-rest potentiation after a 30 s rest is converted to a transient depression. This effect is similar to that seen in the untreated muscle after a very long rest. Each column depicts the mean of 5 experiments. Post-rest contractions after ryanodine are significantly different from the corresponding contractions in the control, $P < 0.01$ level (paired t test).

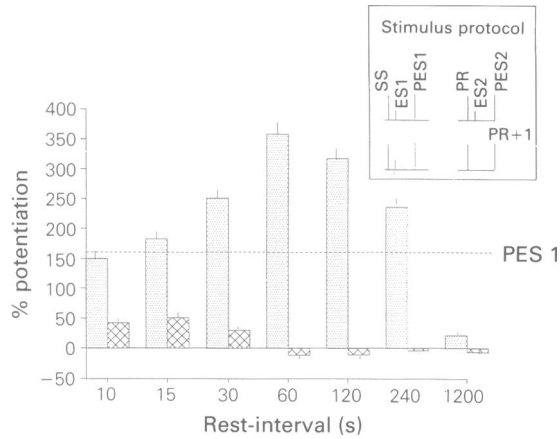

Figure 8 Effect of rest on post-extrasystolic potentiation. The inset illustrates the two experimental protocols. In the top procotol two extrasystoles and their corresponding post-extrasystolic potentiated beats are shown, one before (ES1; PES1) and one after a period of rest (ES2; PES2). The second type of protocol was used to correct for the contribution of residual rest potentiation to the amplitude of PES2. The difference between the observed PES2 and the contribution of decaying rest-potentiation (PR + 1) gives the true magnitude of post-extrasystolic potentiation after rest. Stippled columns, post-rest potentiation; hatched columns, PES2 (corrected). The steady state contraction is referred to as SS. The amplitude of PES1 (dashed line) has been shown for reference. For more details see text.

Comparison of post-extrasystolic and rest-potentiation

Even though the experiments with ryanodine suggested that both post-extrasystolic and rest-potentiations may depend on the integrity of the SR, the quantitative differences in the effect of ryanodine on the two types of potentiation and their different time courses suggested that the mechanism controlling post-extrasystolic and rest-potentiation may be different. Rest-potentiation was more sensitive to the blocking action of ryanodine than was post-extrasystolic potentiation. This was most clearly seen at very low concentrations of ryanodine shown in Figure 5. Rest-potentiation was depressed within 10 min with 30 nM ryanodine and was reduced from 373% of steady state beat to 25% in 70 min. In contrast the post-extrasystolic beat was virtually unchanged at this concentration of ryanodine although, as mentioned earlier, it was blocked at higher concentrations of the alkaloid.

In order to test the difference between rest and post-extrasystolic potentiation further, the experimental protocol shown in Figure 8 was employed. The purpose of this experiment was to see whether after a period of rest, which resulted in potentiation, an extrasystole could also elicit potentiation. Four muscles from different animals were stimulated at a basic cycle length of 2000 ms. After being conditioned with a train of 50 normal stimuli they were provided with an extrasystole (ES1) of varying prematurity. A normally spaced post-extrasystolic beat was produced after the extrasystole (PES1). This was followed by another period of conditioning with a steady train of pulses having a basic cycle length of 2000 ms. This was succeeded by another rest period which varied from 5 s to 25 min. The first post-rest beat (PR) was followed by an extrasystole (ES2) which had an interval exactly equal to ES1 preceding the rest period. The next beat (PES2) had the same relation to ES2 as PES1 had to ES1. One of the problems that this experimental design had to circumvent was that any observed increase in PES2 could be due to the residual potentiation from the post-rest beat (as it takes several beats for rest potentiation to decay). To correct for this a second stimulus protocol was adopted in parallel experiments. After obtaining ES1 different durations of rest were imposed as was done previously. After this the stimulation was resumed but no premature stimulus was presented. The second beat after resumption of stimulation (PR + 1) was indicative of the residual

rest-potentiation remaining after the first post-rest beat. When the potentiation seen in the post-extrasystolic beat (PES2) of the previous stimulus protocol was corrected for the potentiation of PR + 1, a clearer picture of the effect of rest on subsequent post-extrasystolic potentiation was obtained. This was done by using the following formula:

$$\text{Corrected PES2} = [\text{PES2} - (\text{PR} + 1)]/\text{SS}$$

In Figure 8 the post-rest potentiation, PES1 and the corrected PES2 values following different durations of rest are compared. The values shown are expressed as % potentiation. It is shown that the effect of rest on the potentiation of a subsequent beat varied with the rest interval. The small difference between the potentiated beat at 60 and 120 s was not statistically significant ($P > 0.05$). Potentiation fell off significantly at rest intervals shorter than 60 s or longer than 120 s. On the contrary, post-extrasystolic potentiation ((PES2), corrected for rest-potentiation (PR + 1)), was significantly reduced after rest periods of 30 s. It seems therefore that conditions which increase rest-potentiation impair post-extrasystolic potentiation.

Discussion

The present experiments were performed to elucidate the mechanisms underlying the interval-force relation. Of special interest to us was the relative importance of the SL and the SR in frequency, post-extrasystolic and rest potentiation. These experiments made use of ryanodine, an alkaloid which impairs release of Ca from the SR either directly or indirectly as a result of depletion. Persistence of the staircase in the presence of ryanodine gives further support to the importance of Ca influx through the SL in causing the phenomenon. Our conclusions regarding the importance of the SR in determining the amplitude of the first beat after rest are similar to those obtained in the rabbit and rat by several investigators (Bers, 1985; Lukas & Bose, 1986).

Role of sarcolemma and sarcoplasmic reticulum in interval-force relationship studied with ryanodine

Experiments were done with ryanodine, which impairs SR function, to investigate the role of SR in causing twitch potentiation due to alterations in the basic cycle length. The overall purpose of these experiments was to test the contribution of a possible increase in Ca influx from the extracellular space during the extrasystolic beat, its accumulation in the SR and its subsequent contribution to the potentiation of the post-extrasystolic beat. Based on the finding that the extrasystole was larger than the steady state contraction by 3.1% in the presumed absence of SR function (in the presence of ryanodine), it can be said that there is extra calcium entry into the cell which may add to the calcium available for the next (post-extrasystolic) contraction. A rough estimate of this extra amount, based on the pCa-tension curve in skinned cardiac muscle by Fabiato (1985a; Figure 2), is less than 1 μM. The amount of calcium needed for a normal contraction, which is about 40% of a maximally potentiated post-extrasystolic beat, is 5 μM. Had the amount of calcium entering the cell during an extrasystole remained the same as the amount entering during a steady state contraction, the post-extrasystolic contraction should have theoretically remained unchanged. This is because the interval between the extrasystole and the post-extrasystolic contraction remained the same as the interval between two regularly stimulated contractions. However, the additional calcium entering during the extrasystole can account for only 25% of the potentiation, which is less than that observed during post-extrasystolic potentiation. Our calculation of the extra calcium entering the cell during an extrasystole may be an overestimate. It is possible that ryanodine may affect the Ca buffering ability of the SR by impairing the function of the SR. As a result, a greater fraction of the calcium coming from the transmembrane route could participate in the contraction. It seems reasonable to speculate that the extra calcium for extrasystolic potentiation may result from an increase in the mobilization of Ca from an intracellular pool, in addition to the priming of the intracellular pool by the Ca entering the cell during the extrasystole. Two possible explanations may be considered. There may be a fraction of Ca entering the cell during an extrasystole which does not contribute to the immediately occurring beat but is stored in the SR and released during post-extrasystolic potentiation. Another possible explanation is that the rate of recycling of Ca from the uptake to the release site of the SR is interval-dependent. Our data show that mechanical restitution is faster at a shorter basic cycle length (unpublished observations). This is somewhat different from the results of Edman & Johannsson (1976) who showed that, in rabbit papillary muscle, tension recovery after a preceding contraction was maximum after about 800 ms regardless of the 'priming' frequency preceding the test pulse. This points to a frequency-independence of the recycling mechanism. This difference may arise from the fact that the SR is functionally deficient in the rabbit (Bers, 1985). Hence one will see a lesser dependence of mechanical restitution on the previous driving frequency in this species. The present data strongly suggest a

frequency-dependence of Ca influx as a primary event in the interval-force relationship in the dog followed by increased release of Ca from the SR.

Differences between post-extrasystolic and rest-potentiation

Rest potentiation is associated with conditions which are different from those necessary for post-extrasystolic potentiation or frequency potentiation. Some of the experiments described in this paper suggest that the mechanisms of these two types of potentiation are different and the results are consistent with different speeds of 'repriming' of the 'release pool' of Ca in the SR during these two types of potentiation. Post-extrasystolic potentiation is associated with a faster 'repriming' of the release pool in the SR. Consequently if this process is impaired there will be a decrease in the post-extrasystolic potentiation. After rest for 120 s, rest-potentiation is maximal while post-extrasystolic potentiation, elicited immediately after this rest period is at a minimum. This suggests slowing of 'repriming' during rest. Another difference between the two types of potentiation is their response to ryanodine. This can be explained on the basis of the postulated ability of ryanodine to increase 'leak' of Ca from the SR (Hilgeman, 1982). Since there is more time for this process during rest as compared to the post-extrasystolic period, a greater decrease in the size of the post-rest beat is expected. Indeed, the experimental observations support such a prediction.

Model

Our data are consistent with the 'recirculation models' mentioned in the Introduction (Wood *et al.*, 1969; Morad & Goldman, 1973; Kaufmann *et al.*, 1974; Allen *et al.*, 1976; Edman & Johannsson, 1976; Wohlfart & Noble, 1982; Schouten *et al.*, 1987). The action potential causes the release of Ca from an intracellular release site in the SR to cause contraction. Ca entering the cell through both the slow inward current and possibly through the Na-Ca exchange (Coraboeuf, 1974; Horackova & Vassort, 1976) may also be taken up by the SR for release during a subsequent beat. The ability of Ca, coming from these two sources to activate the contractile mechanism is doubtful during normal contractions but may become increasingly important when the action potential is prolonged. Membrane repolarization causes a decrease in Ca entry from the extracellular space. Uptake of Ca by the SR Ca pump causes relaxation. Ca in the SR uptake site may be translocated to the release site physically or functionally, possibly in a frequency-dependent fashion. Ca bound to the inside of the SL is eliminated from the cell by Na-Ca exchange (Glitsch *et al.*, 1970) or a Ca pump, and the process is ready to be repeated. According to this model, frequency potentiation results from (i) an interval-dependent increase in the magnitude of the slow inward current during each beat (Noble & Shimoni, 1981), (ii) an increase in total cellular Ca content as a result of increased Ca influx due to the average increase in the time the SL is depolarized (Braveny & Sumbera, 1970) and (iii) an interval-dependent increase in the rate of recycling of Ca from the uptake to the release site in the SR (Morad & Goldman, 1973) or in the ability of a Ca pool to be released in the SR (Fabiato, 1985a). It will be appropriate to use the term 'repriming' to describe the process governing recovery of tension dependent upon refilling of the release compartment of the SR, either by Ca entering the cell or recirculating back from the uptake sites in the SR and the term 'restitution' to describe recovery of inactivation of the Ca release process after previous activation. Fabiato (1985b) has argued against the postulation of anatomically distinct sites in the SR for Ca uptake and release. This argument is supported by a lack of data consistent with Ca redistribution as seen in the skeletal muscle SR using electron-probe microanalysis (Somlyo *et al.*, 1981). Based on these reasons one ought to propose a functional rather than structural compartmentalization of SR Ca, with the difference between uptake and release based on time- and concentration-dependent activation and inactivation of Ca release from the SR and of Ca accumulation in the SR. The presence of a slow component of Ca increase around the rat cardiac SR results in a positive staircase phenomenon with a decrease in stimulus interval, whereas an absence of this component causes a negative staircase (Fabiato, 1985b). While these results can easily explain frequency potentiation and to some extent post-extrasystolic potentiation, it is difficult to explain rest potentiation in the dog because releasable Ca in the SR continues to build up for 60–120 s during the rest period resulting in an increase in the amplitude of the post-rest contraction. As no action potentials occur during this period, the slow component of Ca rise, necessary for refilling of the SR, is absent. To explain satisfactorily the rest potentiation one will have to invoke a slow process of 'recycling' of Ca from the uptake to the release site or a slow process of 'repriming' of a release pool or 'restitution' of a release mechanism. According to calculations made by Fabiato (1985a) the Ca release process in the rat has an absolute refractory period of 0.8 s and a relative refractory period of 0.8–3.5 s. These time periods are too fast to be compatible with the actual time course of rest potentiation we have demonstrated in the dog. Furthermore, recovery from such an inactivation process

does not explain the large increase in potentiation after rest in the absence of Ca influx from the intracellular space during the preceding rest period, unless a large amount of inactivation is present even in muscles stimulated at a normal rate. The model proposed by Adler *et al.* (1985) attempts to describe a variety of potentiation phenomena dependent on alterations in stimulus intervals. This model is essentially a refinement of older models based on recirculation of Ca in the SR. However, rest potentiation is not explained by this model. The more recent model of Schouten *et al.* (1987) explains rest potentiation in the rat on the basis of Ca translocation from an intermediate compartment in the SR between the uptake and release compartments. This model is more appropriate for explaining our results. We propose that an extrasystole would augment both cellular Ca content and the 'repriming' (recirculation of Ca taken up by the SR) or 'restitution' rate (recovery of inactivation of the Ca release process with time; and assuming that during normal stimulation there is a large amount of inactivation capable of being lost during rest). Finally, the degree of potentiation after rest would depend upon the balance between three factors: (1) the extent of 'repriming' of the SR with Ca or 'restitution' of the Ca release process continuing beyond the previous interstimulus interval, (2) the rate of Ca loss from the release site to some other pool from which it cannot be readily utilized (e.g. the extracellular space), and (3) the rate of decline of the late component of contraction due to the absence of stimulation. One may speculate that the slow decline in tension with increasing duration of rest, which ultimately leads to a rested-state contraction, indicates the decay in the size of the releasable pool in the SR. This situation may be brought about because of a lack of beat-dependent replenishment of the pool and also by a reduction in the frequency-dependent Ca influx process.

It should be noted that the changes in contractions during alteration of the basic cycle length are faster initially in the ryanodine-treated trabecula. This suggests that the interval-dependent sarcolemmal processes reach a new steady state more rapidly than those involving the SR. The latter therefore behaves like a 'flywheel', giving some stability to the rapid changes in Ca movement across the sarcolemma, during changes in heart rate. Indeed, amphibian hearts having relatively sparse SR behave in a manner similar to the ryanodine-treated mammalian heart (Morad & Goldman, 1973; Bose, unpublished observations).

In conclusion, the present work shows that the contribution of extracellular and intracellular Ca to contractile tension varies with the rate and pattern of stimulation. Rapid stimulation as well as premature extrasystole stimulates transmembrane Ca influx which may directly increase the size of the releasable Ca pool in the SR and, in addition, may indirectly increase the release of Ca from the SR either by increasing recycling between the uptake and release site or by some other mechanism which may govern the restoration of the ability of the Ca pool in the SR to be released during excitation. Rest-potentiation occurs because of the continuation of 'repriming' for some time during the initial phase of the rest period, despite the gradual slowing of this process and the lack of action potential mediated Ca influx during the rest period. Replenishment of the release site occurs at a rate faster than the rate of simultaneous loss of Ca to a non-releasable site. Eventually the latter process catches up due to the finite amount of Ca available for 'repriming'. This results in the rested state contraction (Reiter *et al.*, 1978).

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