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Mechanisms of frequency-induced potentiation of contractions in isolated rat atria

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Summary. Mechanisms underlying the potentiation of contractions after periods of high frequency stimulation (post-stimulation potentiation; PSP) and periods of rest (rest potentiation; RP) were investigated in isolated rat atria. Transmembrane action potentials were not changed during PSP and RP and were superimposable upon the pre-test action potentials. However, the ^{45}Ca content of atrial strips was significantly increased during PSP, which indicates a net gain in intracellular Ca. ^{45}Ca content was not changed during RP. PSP and RP were increased in magnitude in atria pre-treated with gallopamil (2.5 μmol/l). This effect was due to a greater depression by gallopamil of the pre-test contractions than the potentiated post-test contractions. In contrast, PSP was abolished in atria exposed to 7.5 mmol/l $[Ca]_o$ and a transient depression of the post-test contractions was seen. RP was also abolished by high Ca medium, but contractions were not depressed after periods of rest. RP, but not PSP, was unmasked when gallopamil was added to high Ca medium to decrease the size of the basal contractions. Conversely, ryanodine (100 nmol/l) abolished RP but did not affect PSP. With ryanodine present, PSP was greatly increased when the extracellular Ca concentration was increased to 5 mmol/l, whereas RP remained abolished. These results suggest that PSP may reflect an increased transsarcolemmal influx of extracellular Ca, possibly mediated through Na-Ca exchange. In contrast, the mechanism suggested for RP is a transient increase in contractile Ca resulting from an intracellular redistribution of Ca to release sites in the sarcoplasmic reticulum.

Key words: Post-stimulation potentiation – Rest potentiation – Excitation-contraction coupling – Sodium-calcium exchange – Ryanodine

Introduction

Excitation-contraction coupling in mammalian cardiac muscle may involve intracellular as well as extracellular pools of calcium, either directly or indirectly. The "contractile calcium" presented to the myofibrils represents a balance of calcium derived from both sources. However, the relative proportions of calcium contributed by each pool vary markedly in different species (Fabiato and Fabiato 1978; Fabiato 1982). Moreover, studies of excitation-contraction coupling are complicated by an inability to control action potential duration (APD) and strength of contraction independently. The APD is very short in rat heart and appears to be relatively independent of stimulation frequency (Coraboeuf and Vassort 1968). Blesa et al. (1970) reported that the APD of rat right ventricular papillary muscle remained constant at stimulation frequencies from 0.1 to 2.5 Hz. Only slight changes in APD have been reported in other studies of rat myocardial preparations in response to wide changes in stimulation frequency (Lazarus et al. 1980; Keung and Aronson 1981; Payet et al. 1981). In contrast, studies in cardiac tissues of other mammals indicate that increases in stimulation frequency produce marked changes in APD (Boyett and Jewell 1978; Boyett and Fedida 1984). Therefore, rat cardiac muscle should be an ideal preparation for investigation of mechanisms of excitation-contraction coupling.

The objective of the present study was to assess the relative contributions of membrane versus intracellular mechanisms in the control of myocardial contractility. Two types of frequency-induced changes in inotropy, which represent perturbations in the excitation-contraction coupling process, were studied in isolated rat atria. Post-stimulation potentiation, the potentiation of contractions after periods of higher frequency stimulation (Whitehorn 1954; Hajdu and Leonard 1961), and rest potentiation, the potentiation of contractions after periods of rest (Benforado 1958), were studied because they are representative of most forms of potentiation seen in cardiac muscle. Isolated atria were used because the atrial action potential is shorter than the ventricular action potential and hence should be more independent of the stimulation frequency.

Methods

Contractile studies were performed on isolated atria obtained from male Wistar rats (250–350 g) anaesthetized with diethyl ether. Hearts were removed from animals and washed in cold Krebs-Henseleit solution. The atria were removed, separated, and opened by a longitudinal incision. The sino-atrial node region was excised from right atria to prevent spontaneous pacemaker activity. Atria were suspended in 15 ml water-jacketed organ baths containing oxygenated Krebs-Henseleit solution maintained at 37° C. Krebs-Henseleit solution had the following composition (mmol/l): NaCl 118.0; KCl 4.7; KH_2PO_4 1.4; $NaHCO_3$ 25.0; $MgSO_4$ 1.2; $CaCl_2$ 2.5 and glucose 11.0. The pH of the final solution was 7.2–7.4 (at 37° C) after gassing with

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95% O_2 – 5% CO_2. Atria were attached at one end to a stimulating electrode and the other end was attached by silk thread to a Grass FT.03C force transducer. Atria were stimulated at a frequency of 0.3 Hz through stainless steel field electrodes. Stimuli were current pulses 5 ms in duration and at voltages 50% above threshold voltage. Stimulation patterns were generated by digital stimulators (Pulsar 4i and 6i; Frederick Haer Co.). Atria were equilibrated for 60 min at a resting tension of 9.8 – 12.4 mN. Resting tension was then adjusted to obtain maximal twitch tension. Resting tension and isometric contractions were recorded on a Grass model 7 Polygraph. Tension tracings were digitized (HiPad; Houston Instruments Inc.) and analysed on a PDP-11/34 computer (Digital Equipment Corp.).

Experiments in which electrical activity was recorded were performed on predominantly right atria. Atria were mounted in a flow-through plexiglass chamber (40 ml volume). Oxygenated (95% O_2 – 5% CO_2) Krebs-Henseleit solution was supplied to the chamber at a rate of 10 ml/min. Atria were pinned at one end to the silicone rubber floor of the chamber, and the other end was attached by silk thread to a force transducer. Stimulation was accomplished through platinum punctate electrodes positioned on the endocardial surface. Stimuli were current pulses 1 ms in duration with voltages 50% above threshold (Pulsar 4i and 6i; Frederick Haer Co.). Atria were equilibrated for 60 min at 0.3 Hz and under an imposed resting tension of 4.9 mN. Intracellular recordings were made from endocardial muscle cells using glass capillary microelectrodes filled with 2.7 mmol/l KCl (20 – 40 MΩ resistances). Microelectrodes, attached to Ag-AgCl wires, were connected to a Neuroprobe amplifier (Transidyne General Corp.). Action potentials, dV/dt and isometric tension were displayed on a Hewlett-Packard 4-channel storage oscilloscope.

Calcium exchange was studied in isolated atrial strips. Atrial strips (3 – 5 mg) were mounted in vertical 20 ml water-jacketed organ baths containing oxygenated Krebs-Henseleit solution maintained at 37° C. Tissues were equilibrated for 60 min under an imposed resting tension of 4.9 mN. Strips were then incubated for 60 min in Krebs-Henseleit solution containing 1.85×10^6 Bq ^{45}Ca per mmol $CaCl_2$ (New England Nuclear). Complete exchange between cellular calcium and ^{45}Ca in the medium has been reported to occur in rat atrial tissue within 60 min (Gerlach and van Zwieten 1969). Atrial strips were stimulated at a basal frequency of 0.3 Hz throughout the exposure to ^{45}Ca medium. Each atrial strip was then subjected to one of four frequency interventions. Test interventions were terminated by plunging the atrial strips into 10 ml of lanthanum solution (0° – 4° C) with the following composition (mmol/l): NaCl 122; KCl 5.9; $MgCl_2$ 1.25; $LaCl_3$ 50; glucose 11 and Tris-maleate 15 (pH 6.8). After 30 min in lanthanum solution, the atrial strips were blotted between sheets of filter paper and weighed. Strips were then digested in 100 µl of concentrated perchloric acid/nitric acid (1:1, v/v) for 12 h at 50° C. Digests were diluted with 900 µl of 40 mmol/l $LaCl_3$ solution and aliquots (100 µl) were added to 10 ml of scintillation medium and counted for 10 min. The results of each determination were converted to apparent content of ^{45}Ca according to the formula:

$$^{45}\text{Ca content (mmol/kg wet wt)} = \frac{\text{D.p.m. in muscle}}{\text{wet wt (kg)}} \times \frac{\text{mmol Ca/l medium}}{\text{D.p.m./l medium}}.$$

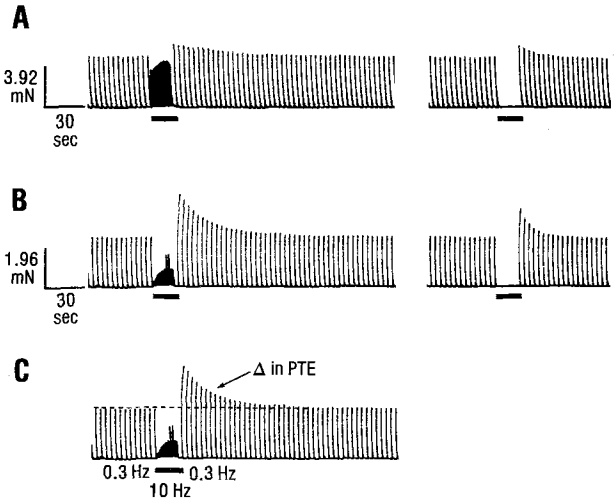

Fig. 1. Potentiated tension responses elicited after a 15 s train of 10 Hz stimulation (post-stimulation potentiation) or equivalent rest duration (rest potentiation) in isolated rat left (*panel A*) and right atria (*panel B*). The atrial preparations were stimulated at a basal frequency of 0.3 Hz. *Panel C:* the change in peak tension envelope (PTE), which was used as an index of the magnitude of post-stimulation potentiation or rest potentiation

Changes in the size of extracellular space were determined during the test interventions using ^3H-polyethylene glycol (McIver and Macknight 1974). Atrial strips were incubated for 60 min in 20 ml of Krebs-Henseleit solution containing 3.7×10^4 Bq ^3H-polyethylene glycol. Tissues were then subjected to basal rate stimulation (0.3 Hz), or stimulation at 10 Hz for 30 s. After termination of the test intervention in lanthanum solution, strips were blotted dry, weighed, and digested in perchloric acid/nitric acid (1:1, v/v) for 12 h at 50° C. Aliquots of the digests (100 µl) were added to scintillation medium and radioactivity was counted for 10 min.

The drugs used in the present study were obtained from the following sources: dl-propranolol · HCl (Sigma Chemicals); sotalol · HCl (Bristol-Meyers); atropine sulfate (Sigma Chemicals); gallopamil (D600; courtesy of Knoll AG, Ludwigshafen, FRG); ryanodine (courtesy of Dr. J. L. Sutko, University of Texas).

Data are expressed throughout as means ± SEM. The statistical significance of single comparisons between atria from the same or different rats was determined using a Student's *t*-test (2 sided). When multiple comparisons were required, an analysis of variance was used in conjunction with a Student-Newman-Keuls test.

Results

1. Post-stimulation potentiation and rest potentiation

Interpolated periods of 10 Hz stimulation or rest caused marked potentiation of the subsequent regular contractions in isolated rat atria (Fig. 1). Ten to fifty potentiated contractions were elicited after a 15 s period of 10 Hz stimulation (post-stimulation potentiation; PSP). The potentiated tension response after an equivalent duration of rest was smaller in magnitude and decayed at a faster rate. Only 5 to 10 potentiated contractions were elicited after a 15 s period

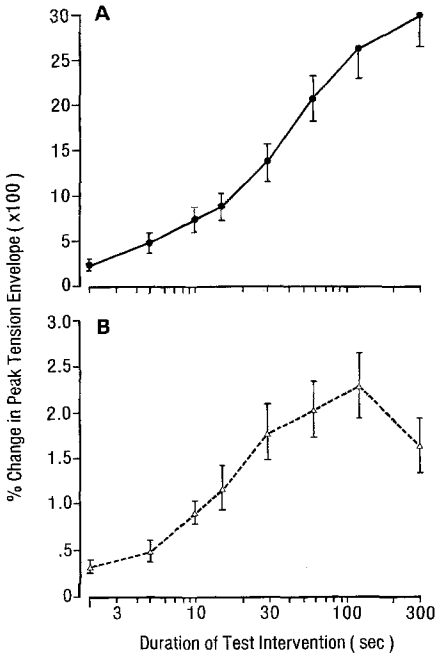

Fig. 2. Effect of the duration of the test intervention on the magnitude of post-stimulation potentiation (*panel A*) or rest potentiation (*panel B*). Right atrial preparations were stimulated at a basal frequency of 0.3 Hz and varying durations of 10 Hz stimulation or rest were interposed. The change in PTE was then measured after each test intervention. Symbols represent the mean ± SE of six preparations

of rest (rest potentiation; RP). PSP and RP were quantified in magnitude by measuring the change in peak tension envelope (ΔPTE) of the potentiated post-test contractions. Three contractions preceding the test intervention were averaged to obtain the pre-test contraction amplitude. This value was subtracted from each potentiated contraction to obtain the ΔPTE in mN (Fig. 1C). The process was repeated until the contraction amplitude returned to the pre-test value. Thus, the ΔPTE was an index of the magnitude of the potentiated tension response elicited by different test interventions. However, to normalize for different pre-test contraction amplitudes, ΔPTE was expressed as a percent of the pre-test contraction amplitude. Zero percent change reflects no potentiation of contractions.

Within the same atrium, periods of high frequency stimulation elicited a greater potentiation of contractions than equivalent rest periods. In 8 right atria, PTE increased by 793.78% ± 150.0 after a 15 s period of 10 Hz stimulation as compared to 117.45% ± 22.4 after a 15 s rest period ($p < 0.001$). In 8 left atria, PTE increased by 284.46% ± 70.6 after a 15 s period of 10 Hz stimulation as compared to 26.93% ± 8.2 after a 15 s rest period ($p < 0.001$). The pre-test contractions in right and left atria averaged 2.88 ± 0.7 mN and 4.69 ± 0.8 mN, respectively. Moreover, the magnitude of PSP and RP was significantly greater in right atria than left atria ($p < 0.05$). Thus, right atrial preparations were used in all subsequent studies.

The magnitude of PSP and RP was markedly dependent on the duration of the test intervention (Fig. 2). As shown in panel A, PSP increased in magnitude as the duration of high frequency stimulation was increased over the range of 2–300 s. In contrast, the relationship between the duration of rest and the magnitude of RP was biphasic. RP increased in magnitude as the duration of rest was increased over the range of 2–120 s (panel B). However, the magnitude of RP decreased progressively after durations of rest exceeding 120 s. The decrease in RP was related to the tension response seen after long periods of rest. In these cases, the initial potentiation of contractions was followed by a subsequent depression of contractions. The relative contribution of each component to the overall response was dependent on the duration of rest. Only potentiated contractions were seen after short periods of rest, whereas fewer potentiated contractions and an increasingly larger number of depressed contractions were obtained after long periods of rest (> 60 s).

The possibility that PSP was due to neurotransmitter release from remnant nerve endings in the atrial preparations was tested in several manners. Table 1 summarizes the effects of point or field stimulation and of receptor blocking agents on PSP and RP. The use of point or field electrodes to stimulate the atria did not significantly alter the magnitude of PSP. Also, the magnitude of PSP and RP was unchanged in the presence of agents that block muscarinic (atropine) or adrenergic receptors (sotalol or propranolol). In Table 1, the mean values within each column were not statistically different from each other. Therefore, PSP is not mediated by neurotransmitter release during high frequency stimulation in isolated rat atria.

2. *Electrophysiological studies*

The electrical and mechanical activity of isolated rat atria during PSP and RP are shown in Fig. 3. A shortening of the action potential duration and a marked reduction in isometric twitch tension occurred during the 15 s train of 10 Hz stimulation (panel B). After reinstitution of the basal stimulation frequency (0.3 Hz), the first seven post-high frequency contractions developed greater tension than control contractions (panel C). No changes in the transmembrane action potentials were detected during the potentiated post-stimulation contractions. The post-stimulation action potentials were superimposable upon action potentials elicited preceding the test intervention. Similar electrical responses were observed during RP (panel D). Interpolation of a 15 s period of rest resulted in potentiation of the first four contractions following the period of rest. Like the post-stimulation response, the potentiated post-rest contractions were not associated with detectable changes in the action potential configuration. The post-rest action potentials were superimposable upon the control action potentials obtained preceding the rest period.

3. *^{45}Ca exchange studies*

Net changes in the transsarcolemmal movement of calcium (Ca) were determined in 6 experiments by measuring changes in ^{45}Ca content during PSP and RP. Right atrial strips stimulated at 0.3 Hz contained 0.2663 ± 0.010 mmol ^{45}Ca/kg wet weight of tissue (Fig. 4). The ^{45}Ca pool detected by this method represents Ca that is not displaceable by lanthanum and presumably is located in the cytosol and intracellular organelles. Stimulation at 10 Hz for 30 s resulted in a small but non-significant decrease in ^{45}Ca content (HFS), which suggests a small loss of Ca by the cells. Also, the size of the extracellular space was not significantly

Table 1. Effects of point and field stimulation and of receptor blocking agents on post-stimulation potentiation and rest potentiation in isolated rat atria

	Right atrium			Left atrium		
	Pre-test force[a]	% Δ in PTE		Pre-test force[a]	% Δ in PTE	
	(mN)	PSP[b]	RP[c]	(mN)	PSP[b]	RP[c]
Field stim.	3.12 ± 0.7	793 ± 150	117 ± 30	4.74 ± 0.8	284 ± 70	26 ± 8
Point stim.	3.02 ± 0.7	851 ± 135	–	4.67 ± 0.8	296 ± 63	–
Atropine (1 μmol/l)	2.73 ± 0.8	901 ± 147	133 ± 27	4.42 ± 0.9	303 ± 49	15 ± 9
Sotalol (1 μmol/l)	3.03 ± 0.6	746 ± 138	104 ± 22	4.71 ± 0.7	240 ± 65	22 ± 6
Propranolol (10 μmol/l)	3.08 ± 0.6	725 ± 116	110 ± 17	4.79 ± 0.8	200 ± 70	30 ± 9

[a] Pre-test contraction amplitude at 0.3 Hz stimulation
[b] Post-stimulation potentiation elicited by 15 s periods of 10 Hz stimulation (mean ± SE, $n = 6$)
[c] Rest potentiation elicited by 15 s periods of rest (mean ± SE, $n = 6$)
PTE: peak tension envelope

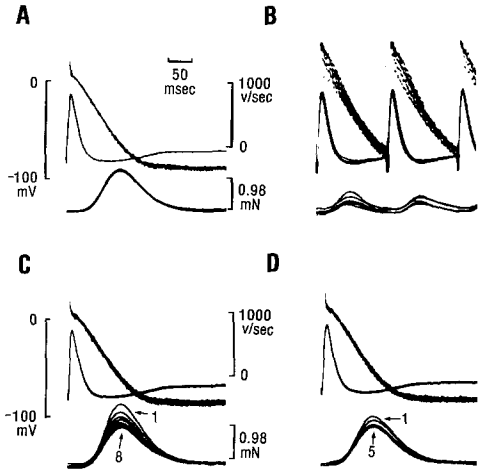

Fig. 3. Changes in transmembrane action potentials and isometric tension during post-stimulation potentiation and rest potentiation in an isolated rat right atrium. In each panel, the lower trace is isometric tension, the middle trace is the first derivative of voltage (dV/dt), and the upper trace is the action potential. *Panel A:* records were obtained during basal stimulation at 0.3 Hz. *Panel B:* records were obtained during a 15 s train of 10 Hz stimulation. *Panel C:* first 8 beats elicited upon return to basal stimulation (0.3 Hz) after high frequency stimulation. *Panel D:* first 5 beats elicited after a 15 s period of rest

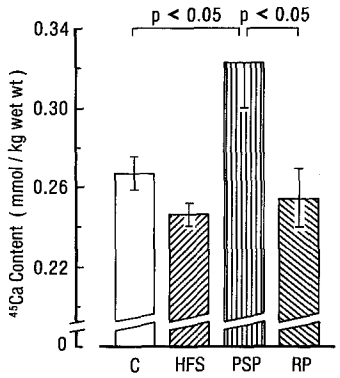

Fig. 4. Changes in ^{45}Ca content measured during post-stimulation potentiation and rest potentiation in isolated rat atrial strips. Right atrial strips were incubated in ^{45}Ca for 60 min and then subjected to one of four frequency interventions. Test interventions were terminated at the following points by plunging the strips into cold lanthanum solution: *C*, control (0.3 Hz stimulation); *HFS*, at the end of a 30 s period of 10 Hz stimulation; *PSP*, after the 2nd potentiated post-stimulation contraction; *RP*, after the 2nd potentiated post-rest contraction. Values represent the mean ± SE of six atrial strips

changed during this period, as determined by tritiated polyethylene glycol (28.34% ± 4.9, Control versus 22.52% ± 6.3, HFS). However, PSP was associated with a significant increase in the ^{45}Ca content of right atrial strips. In these tissues, ^{45}Ca content was determined after the second potentiated post-stimulation contraction. In contrast, ^{45}CO content in atrial strips was not changed during RP. The ^{45}Ca content measured after the second potentiated post-rest contraction was not different from control but was significantly lower than that measured during PSP.

4. *Effect of external [Ca]*

The role of transmembrane Ca fluxes in PSP and RP was further assessed using the calcium channel blocking agent, gallopamil. Table 2 summarizes the effects of gallopamil on PSP and RP in 6 right atria. Exposure to gallopamil (2.5 μmol/l) reduced the basal rate contractions by 30%. However, the amplitude of the potentiated post-stimulation or post-rest contractions was only slightly decreased by gallopamil. Hence, PSP and RP were increased in magnitude due to a greater depression by gallopamil of the pre-test contractions than the potentiated post-test contractions (ΔPTE is expressed as a percent of the pre-test contraction amplitude obtained in the presence of gallopamil). Similar results were also obtained with the inorganic calcium channel blocking agents $MnCl_2$ and $NiCl_2$ (0.5 – 0.75 mmol/l).

The extracellular concentration of Ca ($[Ca]_o$) was increased to 7.5 mmol/l in 6 experiments to stimulate the transsarcolemmal influx of Ca. The effects of 7.5 mmol/l $[Ca]_o$ on PSP and RP are summarized in Table 2 and tension tracings from a typical experiment are shown in Fig. 5. Panel A shows the control tension responses obtained at 2.5 mmol/l $[Ca]_o$. Exposure to 7.5 mmol/l $[Ca]_o$ greatly increased the size of the basal rate contractions (panel B). The tension developed by the high frequency contractions was also increased, and was greater in amplitude than the basal rate contractions in

Table 2. Effects of gallopamil and increased extracellular calcium concentration on post-stimulation potentiation and rest potentiation in isolated rat right atria

Intervention	Pre-test force[a]		% Δ in PTE	
	(mN)	(% control)	PSP[b]	RP[c]
Control	2.98 ± 0.7	100.0	871.3 ± 124	117.5 ± 30
gallopamil (2.5 μmol/l)	2.15 ± 0.7	72.2	1179.8 ± 141	154.1 ± 36
7.5 mmol/l $[Ca]_o$	6.87 ± 0.6	230.5	−65.3 ± 35	5.7 ± 14
7.5 mmol/l $[Ca]_o$ plus gallopamil (50 μmol/l)	2.51 ± 0.6	84.2	−92.3 ± 69	139.3 ± 33

[a] Pre-test contraction amplitude at 0.3 Hz stimulation
[b] Post-stimulation potentiation elicited by 15 s periods of 10 Hz stimulation (mean ± SE, $n = 6$)
[c] Rest potentiation elicited by 15 s periods of rest (mean ± SE, $n = 6$) PTE: peak tension envelope

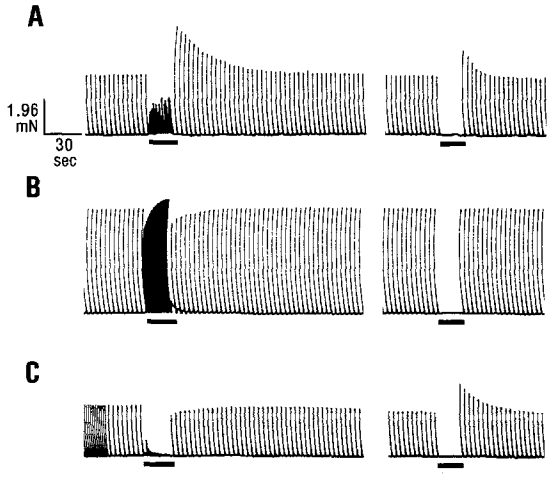

Fig. 5. Effects of calcium-rich Krebs-Henseleit solution, and calcium-rich solution plus gallopamil, on post-stimulation potentiation and rest potentiation in an isolated right atrium. *Panel A*: control tension responses obtained after a 15 s train of 10 Hz stimulation and equivalent duration of rest (2.5 mmol/l $[Ca]_o$). *Panel B*: typical tension responses obtained in the presence of 7.5 mmol/l $[Ca]_o$. *Panel C*: typical tension responses obtained after addition of gallopamil (50 μmol/l) to the calcium-rich solution. Gallopamil decreased the amplitude of the pre-test contractions by about 50%

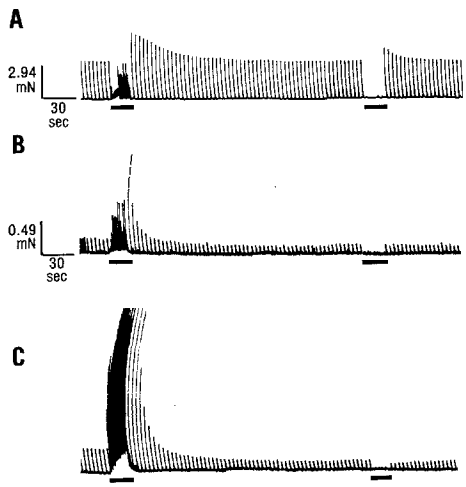

Fig. 6. Effects of ryanodine, and ryanodine plus 5.0 mmol/l $[Ca]_o$, on post-stimulation potentiation and rest potentiation in a right atrial preparation. *Panel A*: control tension responses obtained after a 15 s period of stimulation at 10 Hz and equivalent duration of rest. *Panel B*: typical tension responses obtained 15 min after the addition of 100 nmol/l ryanodine. *Panel C*: typical tension responses obtained in the presence of ryanodine plus 5.0 mmol/l $[Ca]_o$. Note that the top of the force record is cut off in this panel

5 of 6 preparations. However, 7.5 mmol/l $[Ca]_o$ abolished PSP and a transient depression of contractions was seen after the period of high frequency stimulation. Contractile amplitude recovered to pre-test levels in 10–12 beats, but a subsequent potentiation of contractions did not occur. Unlike the post-stimulation response, contractions were not depressed after a period of rest in 7.5 mmol/l $[Ca]_o$ medium. The post-rest contractions were of the same magnitude as the pre-test contractions.

To determine whether abolition of PSP and RP was a primary effect of the high Ca or a secondary consequence of the large increase in basal contractile amplitude, gallopamil (25–75 μmol/l) was added to 7.5 mmol/l $[Ca]_o$ medium to reduce the basal rate contractions by 50%. The effects of gallopamil and 7.5 mmol $[Ca]_o$ on PSP and RP are summarized in Table 2 and a typical experiment is shown in Fig. 5C. In the presence of 7.5 mmol/l $[Ca]_o$, PSP was not unmasked after the basal rate contractions were decreased by gallopamil. Contractile amplitude was depressed for 10–12 beats following the period of 10 Hz stimulation. These effects were not due to gallopamil, since gallopamil alone did not abolish PSP or RP. Therefore, abolition of PSP was due to a direct effect of the high Ca medium. Unlike PSP, RP was unmasked after decreasing the basal rate contractions with gallopamil. Hence, abolition of RP occurred as a secondary consequence to the large increase in basal contractile amplitude induced by the high Ca medium. Similar results were obtained using the inorganic calcium channel blocking agents $MnCl_2$ or $NiCl_2$ (1.0–1.5 mmol/l).

5. *Effect of ryanodine*

Ryanodine is a plant alkaloid that specifically inhibits the release of Ca from the sarcoplasmic reticulum (SR) of cardiac muscle (Sutko et al. 1979; Sutko and Willerson 1980; Sutko and Kenyon 1983). Hence, the relative contribution of release of Ca from the SR to PSP and RP was assessed in 6 experiments using ryanodine (Fig. 6). Panel A shows the control responses obtained in the absence of ryanodine. Exposure to ryanodine (100 nmol/l) for 15 min (panel B) decreased the size of the basal rate contractions by 90–95% (2.82 ± 0.7 mN versus 0.25 ± 0.05 mN). The number of potentiated contractions elicited by 10 Hz stimulation was also decreased by ryanodine (compare panels A and B).

However, although ryanodine decreased the number of potentiated contractions, the amplitude of the potentiated contractions increased markedly with respect to the basal rate contractions. Thus, ryanodine caused a small but non-significant decrease in PSP (825.12% ± 164.4 for ryanodine versus 871.33% ± 182.6 for control). In contrast, exposure to ryanodine completely abolished RP in isolated rat atria. Moreover, the post-rest contractions were depressed in the presence of ryanodine in 3 of 6 atria. More specifically, the first 5–8 contractions after the rest period developed less tension than the pre-test contractions in these preparations. However, contractile amplitude recovered with successive beats.

In six experiments, $[Ca]_o$ was increased to 5.0 mmol/l in an attempt to reverse the negative inotropic effect of ryanodine (Fig. 6C). In the presence of ryanodine plus 5.0 mmol/l $[Ca]_o$, the basal rate contractions were increased by 40 to 50% as compared to ryanodine alone (0.38 ± 0.10 mN versus 0.25 ± 0.05 mN). More importantly, the magnitude of PSP was greatly increased under these conditions. Both the number of potentiated contractions (8–10 versus 5–7) and the magnitude of the potentiated response (2,510.00% ± 204.1 versus 825.12% ± 164.4, $p < 0.001$) were increased in the presence of ryanodine plus 5.0 mmol/l $[Ca]_o$ as compared to ryanodine alone. Unlike PSP, RP remained abolished under these conditions. Moreover, a transient depression of the post-rest contractions was obtained under these conditions. The magnitude of the depression was greater than that obtained in the presence of ryanodine alone (compare panels B and C).

Discussion

The present study suggests that post-stimulation potentiation (PSP) and rest potentiation (RP) occur by different mechanisms and may involve different sources of calcium (Ca) in isolated rat atria. PSP was significantly greater in magnitude than RP following equivalent durations of test intervention (high frequency stimulation or rest, respectively). This difference was not due to the release of neurotransmitters during high frequency stimulation, since the magnitude of PSP was unchanged in the presence of agents that block muscarinic or adrenergic receptors.

Conceivably, the amplitude of contractions could be transiently increased through an increased influx of extracellular Ca, or an increased intracellular release of Ca. An electrogenic influx of Ca into cardiac cells occurs as the slow inward current (I_{si}) of the action potential (Besseau and Gargouil 1969; Mainwood and McGuigan 1975). Hence, increased influx of Ca during I_{si} is one possible mechanism for PSP or RP. In the present study, transmembrane action potentials were unchanged during the potentiated post-stimulation or post-rest contractions and were superimposable upon action potentials elicited preceding the intervention. Furthermore, inhibition of I_{si} with gallopamil (2.5 μmol/l) had little effect on the magnitude of PSP or RP. Gallopamil, at this concentration, causes 40% inhibition of I_{si} in rat myocardium (Payet et al. 1980). Moreover, greatest inhibition of I_{si} would be expected just after the period of high frequency stimulation, since the effects of gallopamil on I_{si} are use-dependent (McDonald et al. 1984). These results suggest that changes in I_{si} are not involved in PSP or RP in isolated rat atria. The present findings are in accord with previous reports that gallopamil does not affect the magnitude of RP in cardiac tissues from other mammals (Lewartowski et al. 1978; Endoh and Iijima 1981).

Another possible mechanism for increased influx of Ca into cardiac cells is through Na-Ca exchange. However, Na-Ca exchange activity could not be measured directly in the present study. Instead, net changes in transsarcolemmal Ca fluxes were assessed from the changes in ^{45}Ca content measured during high frequency stimulation, PSP and RP in atrial strips. Of these frequency interventions, only PSP was associated with a significant change in ^{45}Ca content. An increase in ^{45}Ca content of 76 μmol/kg wet wt tissue was obtained after the 2nd potentiated post-stimulation contraction. This represents a net influx of 38 μmol Ca/kg wet wt per potentiated contraction above the control value. The magnitude of this influx could easily account for PSP through direct activation of the myofilaments (Solaro et al. 1974) or through Ca-induced release of Ca from the sarcoplasmic reticulum (Fabiato 1983). As discussed previously, changes in the transmembrane action potentials were not seen during PSP. Thus, it is unlikely that the increased ^{45}Ca content was due to increased influx of Ca during I_{si}. The only other sarcolemmal system capable of large and rapid movements of Ca is Na-Ca exchange (Reuter 1974; Reeves and Sutko 1979; Langer 1982; Carafoli 1985). However, increased activity of Na-Ca exchange during PSP can only be inferred from the increased ^{45}Ca content.

Unlike PSP, ^{45}Ca content was not changed during RP in rat atrial strips. The ^{45}Ca content measured after the 2nd potentiated post-rest contraction was not changed from control, but was significantly lower than that measured during PSP. Therefore, RP does not involve an increased influx of Ca during the potentiated contractions as may be the case for PSP. The present results are in accord with a previous report by Lodge et al. (1985) in canine ventricular tissue. These authors also reported that ^{45}Ca content was not different from control after the 2nd post-rest action potential.

The extracellular concentration of Ca ($[Ca]_o$) is an important factor in determining the force of cardiac muscle contraction. In isolated rat ventricular tissues, contractile force reaches a maximum at 2–4 mmol/l $[Ca]_o$ (Forestor and Mainwood 1974; Schouten 1984). Somewhat higher Ca levels (5 mmol/l) are required to elicit maximal force in isolated rat atria (unpublished observation). Further increases in $[Ca]_o$ do not increase the force of contraction in these tissues. Hence, maximal force probably reflects saturation of one of the steps in excitation-contraction coupling at $[Ca]_o$ above 5 mmol/l. Therefore, potentiation of contractions would not be expected at high $[Ca]_o$, since contractile force would already be maximal at these Ca levels. Indeed, PSP was abolished at 7.5 mmol/l $[Ca]_o$ in the present study. However, PSP was not restored when the force of contraction was decreased with gallopamil. Na-Ca exchange is not affected by gallopamil (Reeves and Sutko 1980). Thus, only the influx of Ca as I_{si} would be decreased under these conditions. In turn, Ca-induced release of Ca from the sarcoplasmic reticulum (SR) also would be decreased (Fabiato 1983). However, PSP remained abolished under these conditions. These results suggest that saturation by high $[Ca]_o$ of the process of Ca-induced release of Ca from the SR was not responsible for abolition of PSP at 7.5 mmol/l $[Ca]_o$. Instead, the absence of PSP at 7.5 mmol/l $[Ca]_o$ may reflect saturation by high $[Ca]_o$ of the transsarco-

lemmal influx of Ca through a system other than the Ca channel, possibly Na-Ca exchange.

Rest potentiation also was abolished at 7.5 mmol/l $[Ca]_o$. In contrast to PSP, however, RP was present when the basal contractile force was decreased by gallopamil. These results suggest that abolition of RP at high $[Ca]_o$ was due to saturation of the process of Ca-induced release of Ca from the SR. Consequently, RP was restored when the trigger Ca for this process (Ca entry during I_{si}) was decreased to a level below that at which saturation occurred.

The relative contributions of extracellular and intracellular sources of Ca to PSP and RP were also determined using the alkaloid ryanodine. Ryanodine selectively inhibits the release of Ca from SR in cardiac muscle, but does not deplete the SR of Ca (Sutko et al. 1979; Sutko and Willerson 1980; Sutko and Kenyon 1983). The sensitivity of different species to the cardiac actions of ryanodine correlates very well with the sequence of thresholds for Ca-induced release of Ca from SR. The process of Ca-induced release of Ca from SR is the most highly developed in skinned rat atrial cells (Fabiato and Fabiato 1978; Fabiato 1982). Rat myocardium also exhibits the greatest sensitivity to the negative inotropic actions of ryanodine (Sutko and Willerson 1980). Indeed, Chamberlain et al. (1984) have shown that ryanodine blocks Ca-induced release of Ca from purified SR vesicles. Thus, ryanodine eliminates the contribution of Ca release from the SR to tension development. Transsarcolemmal influx of Ca would be the only major source of Ca available to activate the myofilaments under these conditions. Ryanodine does not, however, decrease I_{si} in cardiac tissues (Sutko and Kenyon 1983) or decrease Na-Ca exchange activity (Sutko et al. 1985).

In the present study, ryanodine decreased the twitch tension of isolated rat atria to 10% of control. Similar decreases have been reported with ryanodine in rat papillary muscles (Sutko and Willerson 1980; Bers 1985). Hence, 90% of the Ca involved in normal contraction of rat atria must originate from the SR. Transsarcolemmal influx of Ca may make up the remaining 10%. In contrast to its effects on twitch tension, ryanodine did not significantly affect the magnitude of PSP in isolated rat atria. However, the pattern of PSP was noticeably different after exposure to ryanodine. Fewer potentiated contractions were obtained after ryanodine treatment, but the amplitude of the potentiated contractions was markedly increased. PSP could only occur through increased influx of activating Ca under these conditions, since Ca release from the SR would be eliminated by ryanodine. Consistent with this suggestion, the magnitude of PSP was greatly increased in the present study by conditions that increased the transsarcolemmal influx of Ca (i.e. increased $[Ca]_o$).

Unlike PSP, RP in isolated rat atria was completely eliminated by ryanodine treatment. Ryanodine is also a potent antagonist of RP in rat ventricular tissues (Hajdu 1969; Sutko and Willerson 1980; Bers 1985). Therefore, if ryanodine specifically impairs SR function, then the relative contribution of Ca release from the SR to RP is very large in rat myocardium. Previously, several investigators have suggested that RP depends primarily on the release of Ca from intracellular stores (Hajdu 1969; Allen et al. 1976; Lewartowski et al. 1978). Our results are in accord with this hypothesis. This view is also supported by the findings of Endoh and Iijima (1981) that RP is inhibited by agents such as theophylline and caffeine, which are known to affect the Ca handling of SR in cardiac tissues.

In conclusion, the present study provides evidence that PSP and RP occur by different mechanisms in isolated rat atria. Results from the present study suggest that PSP occurs through increased transsarcolemmal influx of Ca during the potentiated contractions. The mechanism mediating the increased influx of Ca has not been unequivocally identified, but Na-Ca exchange is a likely candidate. Indeed, high frequency stimulation induces a number of ionic changes that would promote increased Na-Ca exchange activity upon return to basal rate stimulation. Large increases in stimulation frequency are known to increase the intracellular concentration of ($[Na]_i$) in cardiac tissues (Cohen et al. 1982). Thus, as $[Na]_i$ rises, the influx of Ca would be increased through Na-Ca exchange, because of competition between Na and Ca for active sites on the exchange carrier (Reeves and Sutko 1983). However, it is not clear why ^{45}Ca content was not incresed during the period of high frequency stimulation in the present study. One possible explanation is that the increased influx of Ca was offset by an increased efflux of Ca during high frequency stimulation, but this remains to be determined. Upon return to basal rate stimulation, the large increase in $[Na]_i$ would continue to promote influx of Ca through Na-Ca exchange during the post-test contractions. As a result, PSP would be obtained through such a mechanism until the $[Na]_i$ was returned to its pre-test level by various cellular processes.

In contrast to PSP, the phenomenon of RP appears to depend primarily on the release of Ca from intracellular stores. Based on available evidence, we propose that the rest period provides a longer interval for the transfer of Ca from uptake to release sites in the SR, thereby increasing the quantity of Ca available for release by the post-rest beats. Moreover, the rapid decay of RP probably reflects depletion of the "extra" Ca in the release sites.

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