Effects of Thapsigargin and Cyclopiazonic Acid on Twitch Force and Sarcoplasmic Reticulum Ca\(^{2+}\) Content of Rabbit Ventricular Muscle

Stéphane Baudet, Rayan Shaoulian, Donald M. Bers

Thapsigargin (TG) and cyclopiazonic acid (CPA) are reported to be specific high-affinity inhibitors of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) pump in isolated membranes and cells, with TG causing complete pump inhibition at nanomolar concentrations. To evaluate the effectiveness of TG and CPA in small multicellular cardiac preparations, we used rapid cooling contractures (RCCs) to assess the SR Ca\(^{2+}\) load. In contrast to observations in single myocytes, TG caused remarkably slow and incomplete SR Ca\(^{2+}\) depletion in multicellular preparations. A 45-minute exposure to 500 \(\mu\)M TG at 30°C and 0.5-Hz stimulation only decreased RCCs by 76±5% (and 100 \(\mu\)M CPA reduced RCCs by 59±10% [mean±SEM]). In contrast, 10 minutes with 20 mM caffeine completely abolished RCCs. This confirms that there was still a caffeine-sensitive pool of Ca\(^{2+}\) in the TG-treated muscle. The time constant of rest decay of RCCs was accelerated by both TG (from 83±18 to 26±6 seconds) and CPA (from 68±11 to 10±5 seconds). This might be expected since Ca\(^{2+}\) leaking from the SR during rest cannot be taken back up as efficiently, favoring Ca\(^{2+}\)extrusion by the sarcosomal Na\(^+-\)Ca\(^{2+}\) exchanger. TG and CPA decreased twitch force (by 44±7% and 40±11%, respectively) and increased twitch duration, presumably because of the SR effects. We conclude that complete blockade of SR Ca\(^{2+}\) uptake by TG or CPA in multicellular preparations cannot be assumed, even at high [TG] or [CPA], unless evaluated (eg, by RCC). (Circ Res. 1993;73:813-819.)

KEY WORDS • sarcoplasmic reticulum • Ca\(^{2+}\)-ATPase • excitation-contraction coupling • rapid cooling contractures

Ventricular muscle contraction is activated by Ca\(^{2+}\) influx through the sarcolemma, which subsequently releases Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) via the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism.\(^1\)\(^-\)\(^2\) Relaxation is brought about by Ca\(^{2+}\) dissociation from the contractile proteins, extrusion of that Ca\(^{2+}\) by the sarcosomal Na\(^+-\)Ca\(^{2+}\) exchanger, and reuptake by the SR Ca\(^{2+}\)-ATPase.\(^2\)\(^-\)\(^4\) The SR Ca\(^{2+}\) load is an important factor in control of cardiac muscle contraction and is itself determined by a balance between Ca\(^{2+}\) efflux through the SR Ca\(^{2+}\) release channel and Ca\(^{2+}\) uptake via the SR Ca\(^{2+}\)-ATPase.\(^2\)\(^-\)\(^6\)

Until recently, caffeine and ryanodine have been the main agents used to inhibit SR function in intact cardiac cells. Although this approach has been helpful in showing that contraction could be supported to various extents by sarcosomal Ca\(^{2+}\) influx in different animal species,\(^7\) these agents activate SR Ca\(^{2+}\) release rather than inhibiting the SR Ca\(^{2+}\) pump. This could be a particular problem when using ryanodine, since the SR can still accumulate Ca\(^{2+}\), albeit transiently.\(^8\) An additional impact that SR Ca\(^{2+}\) uptake inhibition would have on contractile force is to alter the Ca\(^{2+}\)-buffering role of the SR. In fact, the SR Ca\(^{2+}\) pump can limit the ability of Ca\(^{2+}\) influx to reach the myofilaments.\(^9\) An agent that inhibits the SR Ca\(^{2+}\) pump should block this SR action.

Recently, two chemically unrelated compounds, thapsigargin (TG) and cyclopiazonic acid (CPA), have been used as specific inhibitors of SR/endoplasmic reticulum (ER) Ca\(^{2+}\)-ATPase. TG or CPA can induce Ca\(^{2+}\) loss from the SR/ER. Indeed, in cell types that don't have powerful Ca\(^{2+}\) extrusion mechanisms, these agents cause [Ca\(^{2+}\)] to rise.\(^10\)\(^-\)\(^11\) In cardiac and skeletal muscle SR vesicles, TG and CPA inhibit SR Ca\(^{2+}\) uptake at nanomolar concentrations. In isolated cardiac myocytes, TG decreases cell shortening by progressively depleting the SR of its Ca\(^{2+}\) but does not appear to affect SR Ca\(^{2+}\) release (when the SR contains Ca\(^{2+}\)) or the L-type Ca\(^{2+}\) current at the concentrations used.\(^12\)\(^-\)\(^14\) Therefore, these two compounds could be valuable tools in the study of the impact of SR Ca\(^{2+}\) pump inhibition on contractile force and relaxation in multicellular cardiac preparations. However, no studies have tested the ability of TG or CPA to prevent SR Ca\(^{2+}\) uptake in such multicellular cardiac preparations.

It is difficult to directly assess the SR Ca\(^{2+}\) content in intact functioning cardiac muscle. Rapid cooling contractures (RCCs) have been found to provide a valuable index of SR Ca\(^{2+}\) content in mammalian cardiac muscle.\(^2\)\(^,\)\(^5\)\(^-\)\(^8\)\(^,\)\(^15\)\(^-\)\(^19\) Rapidly cooling the cardiac muscle to 0°C appears to cause release of all of the SR Ca\(^{2+}\) while simultaneously inhibiting the Ca\(^{2+}\) transport systems.
(eg, SR Ca\(^{2+}\) pump and Na\(^+\)-Ca\(^{2+}\) exchange). The result is a slowly developing contracture (at 0°C), where the amplitude is indicative of the SR Ca\(^{2+}\) content at the moment of cooling. Although caffeine can also be used to release SR Ca\(^{2+}\), it is much less useful in multicellular preparations than in isolated cells because caffeine diffuses slowly into the muscle (and doesn’t slow Na\(^+\)-Ca\(^{2+}\) exchange). Thus, the caffeine-induced contractures in the many cells in a muscle are asynchronous, with cells at the outside contracting and relaxing (because of Na\(^+\)-Ca\(^{2+}\) exchange) before cells in the core are activated.

The aims of the present study were to determine, in multicellular preparations, the effects of TG and CPA (1) on SR Ca\(^{2+}\) accumulation using RCCs as an indicator of SR Ca\(^{2+}\) load, (2) on steady-state twitch parameters, and (3) on the balance of Ca\(^{2+}\) fluxes between the Na\(^+\)-Ca\(^{2+}\) exchanger and the SR Ca\(^{2+}\) pump during rest.

**Materials and Methods**

**Muscle Preparations**

The protocol for muscle dissection, mounting, and RCCs has been previously described.\(^5\) In brief, thin papillary muscles or ventricular trabeculae (0.1 to 0.7 mm in diameter) were obtained from the hearts of New Zealand White rabbits previously anesthetized with pentobarbital sodium (40 mg/kg IV). Each muscle was tied at both ends with a fine nylon thread; one end was fixed, and one end was attached to a force transducer (model AE 875, SensoNor, Horten, Norway; or Kulite Semiconductor, Leonia, NJ). Muscle shape was assumed to be cylindrical, and diameter was measured by means of a micrometer in the dissecting microscope. Force was expressed in millinewtons per square millimeter.

The solutions fed the chambers by gravity (flow rate, \(\approx 25 \text{ mL/min}\)) and were maintained at the desired temperature by water jacketing at 30°C or by jacketing by a mixture of polypropylene glycol and water (1:3) at \(-4°C\). Solenoid pinch valves, at the bath inlet, were gated by a stimulator, allowing synchronized solution changes. At this flow rate, switching to the cold solution cools the surface of the muscle to below 5°C in less than 200 milliseconds, and rewarming to 30°C is similarly rapid. Transducer signals were digitized at 500 Hz using Axotape software (Axon Instruments, Foster City, Calif).

**Solutions**

Muscles were superfused with a modified Tyrode’s (NT) solution containing (mM) NaCl, 140; KCl, 6; MgCl\(_2\), 1; CaCl\(_2\), 2; glucose, 10; and HEPES, 5 (pH 7.4 at 30°C). Solutions were gassed with 100% \(O_2\). Stock solutions of TG (5 mM) and CPA (5 or 30 mM) were prepared in dimethyl sulfoxide and kept in a freezer until use. Our experience has shown that some precautions must be taken with TG. First, according to Calbiochem Corp, La Jolla, Calif, this molecule is light sensitive, so the experiments were performed in the dark. Second, TG may be sensitive to oxidation, so that bubbling the TG-containing solutions with \(O_2\) was avoided. Lack of \(O_2\) bubbling by itself (without TG) did not alter RCC amplitude in control experiments. Finally, TG may adhere to the tubing.\(^{20}\) Therefore, after every experiment, all the perfusion lines were washed with alcohol and several liters of deionized water. Such problems do not seem to exist with CPA, but the same precautions for washing the lines were used.

**Experimental Protocol**

After equilibration (\(\approx 90\) minutes), at a stimulation frequency of 0.5 Hz, steady-state twitch tension was recorded, and an RCC was elicited in place of a twitch, to estimate relative SR Ca\(^{2+}\) content under control conditions. To conserve TG and CPA, and also to limit tubing exposure, TG or CPA at the final concentrations in NT solutions was added directly in the bath via a Pasteur pipette. Then the bath was flushed with excess solution with the same drug concentration every 5 minutes along with the bath flow and drain stopped. During these no-flow conditions, stimulation was stopped. Thus, we have been less able to follow the time course of action of the drugs. Drug exposure was for 45 minutes, and then the flow of drug-free Tyrode’s solution and recirculation were restored. This was possible because, at the high drug concentrations used (500 \(\mu M\) TG and 100 \(\mu M\) CPA), their effects on twitch force and RCC amplitude were irreversible. That is, even though TG did not abolish SR Ca\(^{2+}\) uptake, the block was stable, with no recovery for more than 3 hours after TG washout. Pilot experiments (only NT applied with flow stopped for 45 minutes) and vehicle control experiments (up to 10% dimethyl sulfoxide) have shown that 20 minutes was usually necessary for the muscle to recover. Often the twitch amplitude was slightly depressed (by less than 10%), but more importantly, the SR Ca\(^{2+}\) content, assessed by the RCC amplitude, was unchanged, allowing us to test the effects of TG and CPA.

Finally, we tested the ability of caffeine (20 mM) and ryanodine (10 \(\mu M\)) to inhibit the RCC in TG- or CPA-treated muscles. The TG- or CPA-treated preparations were exposed to caffeine or ryanodine until twitch force reached a new steady state (\(\approx 10\) to 20 minutes). Because the effects of ryanodine were irreversible, these effects were tested only after treating the muscle with either of the compounds.

**Chemicals and Statistics**

All salts, caffeine, and CPA were obtained from Sigma Chemical Co, St Louis, Mo. Caffeine was dissolved directly in NT. TG was obtained from Calbiochem. Ryanodine (stock solution of 10 mM in distilled water) was obtained from Agri Systems Corp, Wind Gap, Pa, and from Calbiochem. CPA and TG stock solutions were kept in a freezer. Results are expressed as mean±SEM. Paired and unpaired Student’s \(t\) tests were used when appropriate. A value \(P<.05\) was considered statistically significant.

**Results**

The value of TG and CPA in physiological experiments is that they can completely abolish SR Ca\(^{2+}\) uptake. Our first experiments were carried out to examine the conditions required to achieve complete SR Ca\(^{2+}\) pump inhibition. If the SR Ca\(^{2+}\) pump is completely blocked, then in the steady state the SR should be depleted of Ca\(^{2+}\). We have therefore used RCCs to assess the SR Ca\(^{2+}\) content.

Although we did not perform systematic dose-response curves for both compounds, we found that RCC amplitude was not strongly affected by concentrations up to 20
μM for CPA and TG. Indeed, at 1 and 50 μM TG, RCC amplitude was only decreased by 29±7% (n=6) and 57±8% (n=6), respectively. These observations were rather surprising, particularly for TG, because of the high affinity of the drug in isolated membrane systems and its capacity to inhibit the intracellular Ca²⁺ transient and contraction in isolated cardiac cells at concentrations less than 5 μM. In isolated rabbit cells, the caffeine-induced contracture (a test for SR Ca²⁺ content) is consistently abolished by 5 μM TG. In an effort to abolish SR Ca²⁺ uptake completely, TG and CPA concentrations have been increased to 500 and 100 μM, respectively.

Even at 500 μM TG, the steady-state RCC was only partially inhibited (by 76±6%; range, 48% to 100%). This surprising result could have been related to the size of the muscle (eg, if there were diffusion limitations, oxidation, or partitioning of TG). However, when the percent depression of the steady-state RCC was plotted against muscle diameter (0.33±0.05 mm for the TG group and 0.38±0.06 mm for the CPA group) or cross-sectional area, no correlation was found (P>.05, not shown). Another possibility was that the total amount of drug present in the bath was insufficient to saturate all the SR Ca²⁺ pump binding sites. A rough calculation shows that it is not likely to be the case. In fact, if the number of SR Ca²⁺ pumps is approximately 6 μmol/kg wet wt and if the muscle has a mass of 2 mg in the worst case [ie, π·r²·length·density = π·(0.035 cm)²·(0.5 cm)·(1.06 g/cm³)], the total number of binding sites is 12 pmol. In 0.1 mL of a 500 μM drug-containing solution, there are 50000 pmol TG in the bath, which was refreshed every 5 minutes. Thus, TG outnumbers the potential binding sites by more than three orders of magnitude.

**Characteristics of the Steady-State RCC**

Figure 1 illustrates the effects of TG on the steady-state RCC in two different muscles (similar qualitative results were obtained with CPA). In the control situation (Fig 1, A and D), cold NT was applied until RCC reached a steady level. After the muscle had been treated with 500 μM TG (Fig 1, B and E), the steady RCC amplitude was depressed by 64% and 41%, respectively. The sharp increase in force upon rewarming is attributed to the rapid increase in myofilament Ca²⁺ sensitivity with increasing temperature. In some muscles, the rewarming spike persisted, despite the abolition of the RCC in CPA- or TG-treated muscles (not shown). This indicates that there was still Ca²⁺ left in the SR but not enough to activate the contractile proteins during the cold (since cold decreases myofilament Ca²⁺ sensitivity). Overall, RCCs were depressed by 76±6% (range, 48% to 100%; n=9) by 500 μM TG and 59±10% (range, 36% to 100%; n=7; P>.05) by 100 μM CPA. This variability was not due to variability in muscle diameter (above) or an incapacity of the RCC to be completely abolished, because depletion of SR Ca²⁺ using either caffeine (20 mM) or ryanodine (10 μM) completely abolished both the RCC and the rewarming spike (Fig 1, C and F, respectively). This confirms that the RCC in the presence of TG or CPA represents residual SR Ca²⁺. When 20 mM caffeine was first applied (between Fig 1, B and C), there was usually a transient increase in diastolic force. This was probably due to release (and extrusion) of residual SR Ca²⁺ in the presence of TG upon the application of caffeine.

**Rewarming Spike Relaxation**

In previous studies, the relaxation phase of the rewarming spike has been considered to reflect the rate of decline of [Ca²⁺] due to SR Ca²⁺ uptake and Ca²⁺ efflux through the Na⁺-Ca²⁺ exchanger. Since the SR Ca²⁺ pump is dominant (by a factor of threefold to fourfold), we expect TG and CPA to slow relaxation of the rewarming spike. Since TG and CPA reduce RCC amplitude, it is important for this comparison that changes in the time to half-relaxation (t₁/₂) of the rewarming spike are independent of the amplitude of the RCC. This appears to be the case (not illustrated; see also Bers and Bridge). In CPA-treated muscles, the t₁/₂ of relaxation was consistently slowed, from 355±31 to 521±48 milliseconds (P<.02). In TG-treated muscles, the t₁/₂ of relax-
When the peaks were normalized (Fig 2, C), it is apparent that the time to peak force (TTP) was increased but that t_{1/2} was not appreciably changed. Overall, steady-state twitch force was decreased by 44±7%, from 19.5±2.2 mN·mm⁻² (n=10). TTP was significantly increased by TG (from 284±11 to 362±17 milliseconds, P<.01), but t_{1/2} was unaffected (237±13 milliseconds in the control condition and 238±12 milliseconds after TG).

Fig 2, B and D, illustrate representative effects of 100 μM CPA on the twitch. CPA clearly prolongs the twitch time course by increasing both TTP (by 63%) and t_{1/2} (by 43%). On the average, TTP increased significantly (from 295±12 to 492±21 milliseconds, P<.001) as did t_{1/2} (from 220±9 to 354±15 milliseconds, P<.001). Twitch force was also decreased by 40±11%.

Effects of TG on Rest Decay of Twitches and RCCs

In rabbit ventricular muscle, the amplitude of the first twitch or RCC decreases after increasing periods of rest (a process known as rest decay).⁷,²² During rest, Ca²⁺ leaks from the SR and is either taken up by the SR or extruded through the Na⁺-Ca²⁺ exchanger. Ca²⁺ reaccumulated by the SR does not contribute to rest decay. The ability of the Na⁺-Ca²⁺ exchange to compete with SR Ca²⁺ uptake determines the rate of rest decay of RCCs in rabbit ventricle.¹⁹ Therefore, if TG and CPA were effectively inhibiting Ca²⁺ uptake, rest decay of RCCs should be accelerated, because the balance of Ca²⁺ fluxes will be even more in favor of the efflux. Although rest decay of Twitches is also observed, it is complicated by the contribution that transsarcolemmal Ca²⁺ fluxes have during the postrest twitch (eg, via the L-type Ca²⁺ current).

Fig 3, A, illustrates the effect of TG on the amplitude of the first postrest contraction (ie, the time course of rest decay) normalized to the control twitch. In control conditions, the rest decay was typical for rabbit ventricular muscle. After TG treatment, the steady-state twitch was depressed by 47%, and rest decay was accelerated. The time constant for this rest decay (τ) decreased from 72±11 to 13±1 seconds after TG. To confirm that this was actually due to a faster decline in SR Ca²⁺ content, RCCs were elicited at steady state and after different rest periods (Fig 3, B). TG treatment decreased the steady-state RCC by 76% and also accelerated rest decay of RCCs (τ decreased from 83±18 to 26±4 seconds).

Results obtained with CPA were not qualitatively different from those obtained with TG, and a typical example is shown in Fig 4. CPA accelerated rest decay of both Twitches and RCCs. On the average (n=7), CPA decreased τ from 30 to 13 seconds for Twitches and from 68±11 to 10±5 seconds for RCCs. In Fig 4, ryanodine was also added after equilibration and testing with CPA alone. Ryanodine accelerated the rest decay even more, as may have been expected (⁰=5 seconds). However, the twitch after a 5-minute rest was not further reduced by ryanodine. Thus, this "rested state" twitch (with CPA alone) may reflect activation by Ca²⁺ influx alone.

Discussion

Our results show that TG and CPA both decreased twitch force by decreasing the amount of Ca²⁺ inside the SR (assessed by RCCs), presumably due to partial
inhibition of SR Ca\textsuperscript{2+} uptake. However, despite high concentrations of TG or CPA, the inhibition of the SR Ca\textsuperscript{2+} pump was incomplete. CPA appears to have the expected effects on twitch time course (especially a slowing of relaxation). Whereas TG slowed the overall contraction, it had surprisingly little effect on the \( t_{1/2} \) of relaxation. Finally, acceleration of rest decay of twitches and RCCs shows that, when SR Ca\textsuperscript{2+} uptake is inhibited, Ca\textsuperscript{2+} fluxes during rest are shifted toward Ca\textsuperscript{2+} extrusion by the sarcolemmal Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger.

**Incomplete Inhibition of SR Ca\textsuperscript{2+} Uptake: Possible Mechanisms**

Both TG and CPA inhibit the SR Ca\textsuperscript{2+}-ATPase, although by different mechanisms.\textsuperscript{23,24} The most surprising observation of this study has been the need to apply high concentrations of TG and CPA to observe a significant, but still incomplete, decrease of SR Ca\textsuperscript{2+} content (assessed by RCCs). In fact, in single rabbit ventricular myocytes in the same lab, SR Ca\textsuperscript{2+} uptake is completely blocked, and the SR can be depleted of its Ca\textsuperscript{2+} by a 90-second exposure to 5 \( \mu \)M TG.\textsuperscript{12,25} We have also found that RCCs in single rabbit ventricular myocytes (and accompanying intracellular Ca\textsuperscript{2+} transients) can be completely abolished by a 2-minute exposure to 5 \( \mu \)M TG (J.W.M. Bassani and D.M. Bers, unpublished observations). This concentration of TG had almost no effect on twitch force and RCC amplitude in our preliminary experiments with multicellular preparations even after 1 hour of equilibration. This emphasizes the difference between the ability of TG to inhibit the SR Ca\textsuperscript{2+} pump in isolated myocytes vs multicellular preparations. It also shows that the lack of complete SR Ca\textsuperscript{2+} pump inhibition in multicellular preparations is due to the preparation and not the method used to assess SR Ca\textsuperscript{2+} (ie, RCCs).

As shown by our simple calculation, the number of potential binding sites is outnumbered by the amount of TG and CPA present in the bath by at least three orders of magnitude. Thus, there is plenty of ligand available. The possibility of high nonspecific binding is unlikely,
because titration experiments with permeabilized myocytes matched the expected high number of pump sites.25 Although there are other nonmyocyte binding sites (endothelial, neuronal, and smooth muscle cells), they would not be expected to exceed the concentration in myocytes and would be included in the number of sites expected in homogenates.21

Another possibility, linked to the observation of incomplete inhibition of SR Ca\(^{2+}\) uptake, is that the actual concentration of TG or CPA reaching its binding site is much lower than in the bath. For this to occur, TG or CPA could be degraded (or oxidized), creating a concentration gradient, which would be worsened by elevated muscle diameter. However, we did not find any relation between RCC inhibition and muscle diameter. Therefore, we have no satisfactory explanation for the high drug concentrations needed to inhibit the SR Ca\(^{2+}\) pump. Whatever the reason for the incomplete SR Ca\(^{2+}\) uptake depression, it is important to assess SR Ca\(^{2+}\) content directly in multicellular preparations before assuming that SR Ca\(^{2+}\) uptake is completely abolished.

Another important point in this characterization was to know whether the residual RCC observed with TG and CPA could be prevented by compounds known to fully inhibit the RCC in control conditions. The SR Ca\(^{2+}\) can also be depleted by caffeine and ryanodine, which keep the SR Ca\(^{2+}\) release channels opened, thereby preventing SR Ca\(^{2+}\) accumulation.8 Caffeine and ryanodine both abolished the RCC in the absence or presence of TG or CPA, confirming that the SR Ca\(^{2+}\) released by RCC after TG or CPA treatment was still caffeine and ryanodine sensitive.

Effects of TG and CPA on the Steady-State Twitch Amplitude

It is likely that the decrease in twitch force (by ≈45% with both compounds) was primarily due to a decrease in the SR Ca\(^{2+}\) content (ie, RCCs were reduced by 76% and 59% for TG and CPA, respectively). However, ryanodine, which completely abolishes RCCs, only decreases twitches in rabbit ventricle by 20% to 30% (with Ca\(^{2+}\) influx supporting the contraction).2 Since the SR can still transiently accumulate Ca\(^{2+}\) entering during a twitch with ryanodine,8 it is somewhat surprising that TG and CPA were more effective at inhibiting twitches (even without fully inhibiting the SR Ca\(^{2+}\) pump). Thus, at these high concentrations, TG and/or CPA could have additional effects that decrease twitch force independent of their effects on the SR Ca\(^{2+}\) pump.

One possibility is that myofibrillar Ca\(^{2+}\) sensitivity is decreased. Although Ca\(^{2+}\) responsiveness was unaffected by ≈1 \(\mu\)M TG,13 nothing is known of the effect at higher concentrations. CPA has been shown to increase myofibrillar Ca\(^{2+}\) sensitivity of cardiac muscle fibers.26 Another possibility that could explain the discrepancies is if the L-type Ca\(^{2+}\) current were depressed. At low TG concentrations, the amplitude of the L-type Ca\(^{2+}\) current is unchanged.13 Nothing is known of the effect at higher TG concentrations, but 100 \(\mu\)M CPA decreases Ca\(^{2+}\) current in rat ventricular cells (W. Balke, personal communication). Thus, nonspecific effects of high TG and CPA concentrations could complicate their use in multicellular preparations. On the other hand, the comparison with ryanodine effects may not be entirely fair, because ryanodine can prolong action potential duration and this could help to minimize the negative inotropic effect of this agent.

Effects of TG and CPA on the Time Course of the Isometric Twitch

CPA increased the time to peak twitch tension and \(t_{1/2}\) of relaxation of twitches and RCCs, consistent with partial inhibition of the SR Ca\(^{2+}\) pump.27 TG also increased TTP but did not slow relaxation. The absence of relaxation slowing with TG may indicate that other mechanisms compensate for the partial block of the SR Ca\(^{2+}\) pump at these high TG concentrations. Possibilities could include TG stimulation of Ca\(^{2+}\) efflux by the Na\(^{+}\)-Ca\(^{2+}\) exchanger or an increased rate of Ca\(^{2+}\) dissociation from the myofilaments (eg, a decreased myofilament Ca\(^{2+}\) sensitivity). However, the fact that the contractile characteristics were stable for 3 hours after TG washout would make complicating lower-affinity effects at high TG concentrations seem less probable (ie, low-affinity effects would be expected to be more rapidly reversible).

Effects of TG and CPA on Ca\(^{2+}\) Fluxes During Rest

In rabbit ventricular muscle during rest, Ca\(^{2+}\) leaks from the SR, probably through the SR Ca\(^{2+}\) channel.28 Part of this Ca\(^{2+}\) is extruded by the Na\(^{+}\)-Ca\(^{2+}\) exchanger (which leads to rest decay by decreasing SR Ca\(^{2+}\) content), and most of the rest is pumped back inside the SR (which, by contrast, slows rest decay). Therefore, the amount of Ca\(^{2+}\) inside the SR reflects the balance between these two transporters (sarcosomal Na\(^{+}\)-Ca\(^{2+}\) exchanger and SR Ca\(^{2+}\) pump).6,19 According to this scheme, any drug that inhibits SR Ca\(^{2+}\) reuptake or increases SR Ca\(^{2+}\) release during rest should accelerate rest decay, because the probability of the Ca\(^{2+}\) leaking from the SR being extruded by the Na\(^{+}\)-Ca\(^{2+}\) exchanger is higher. Ryanodine, which does not inhibit the SR Ca\(^{2+}\) pump, is known to take up greatly accelerate rest decay, simply because the Ca\(^{2+}\) taken up almost immediately leaks from the SR to be extruded. In this case, much more Ca\(^{2+}\) leaks from the SR per unit time and is available for extrusion by Na\(^{+}\)-Ca\(^{2+}\) exchange. Our results show that TG and CPA have the expected accelerating effect on rest decay of twitch force and RCC, as indicated by the smaller time constant.

Conclusions

Our results have shown that both TG and CPA decreased the amplitude of twitches and RCCs (and hence, SR Ca\(^{2+}\) content). However, SR Ca\(^{2+}\) uptake depression by TG and CPA is incomplete, even at very high concentrations and long exposure times, which contrasts with more isolated preparations like single cells or SR vesicles. The SR Ca\(^{2+}\) pump inhibition can probably explain the observed decrease of twitch force, but these agents might have other complicating effects at the high concentrations required. Both agents appear to shift the balance of Ca\(^{2+}\) fluxes in favor of Ca\(^{2+}\) extrusion by the sarcosomal Na\(^{+}\)-Ca\(^{2+}\) exchanger, thereby accelerating the rest decay of twitches and RCCs. Finally, CPA appears to have the more expected effect in that it significantly slows relaxation. In any event, the utility of TG and CPA in multicellular cardiac preparations may be seriously limited by incom-
plete effectiveness, particularly if no test for SR Ca$^{2+}$ load is performed.

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