Cardiac contractility modulation by non-excitatory currents: Studies in isolated cardiac muscle

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Abstract

Background: Myocardial contractility can be altered using voltage clamp techniques by modulating amplitude and duration of the action potential resulting in enhanced calcium entry in the cell of isolated muscle strips (Non-Excitatory Currents; NEC). Extracellular electrical stimuli delivered during the absolute refractory period (Cardiac Contractility Modulation; CCM) have recently been shown to produce inotropic effects in-vivo.

Aim: Understanding the cellular mechanism, underlying the CCM effect, is essential for evaluating its clinical potential. We tested the hypothesis that NEC and CCM modulate contractility via similar cellular mechanisms.

Methods: Square wave electric currents were applied in the organ bath to isometrically contracting rabbit RV papillary muscle and human failing trabecular muscle during the absolute refractory period (ARP).

Results: These currents, which did not initiate new action potentials or contractions, modulated action potential duration (shortened or lengthened) and contractility (enhanced or depressed) in a manner that depended upon their amplitude, duration and delay from the pacing stimulus. The contractility modulation effect in the rabbit RV papillary muscle was markedly blunted after exposure to ryanodine, indicating that the sarcoplasmic reticulum plays an important role in the contractility modulation.

Conclusion: Like voltage clamping, extracellular currents applied during the ARP can similarly modulate action potential duration in-vitro and modulate myocardial contractility by similar intracellular mechanisms. This concept provides the potential of a therapeutic strategy in patients with heart failure to enhance contractility.

Keywords: Inotropy; Sarcoplasmic reticulum; Papillary muscle; EC coupling; Membrane potential

1. Introduction

Despite advances in therapies for heart failure, consisting of diuretics, digoxin [1], ACE Inhibitors [2], aldosterone inhibitors [3,4] and beta-blockers [5,6], which have significantly improved survival and quality of life, mortality and morbidity remain relatively high [6–8]. Accordingly, investigation of novel therapies to treat these patients, particularly with the goal of improving exercise tolerance and quality of life, is a high priority. Cardiac resynchronization (CRT) is among the most important of the recently studied treatments proven to provide such benefits to patients with NYHA class III and IV symptoms [9,35,36]. However, CRT is only applicable to patients with abnormal activation sequence manifest as a prolonged QRS durations, which is present in less than half of patients. Availability of a device that is easily implantable that could provide similar benefits to patients with normal activation sequence (i.e., normal QRS duration) would have a significant impact.

Reduced calcium delivery to myofilaments is believed to contribute to contractile dysfunction in heart failure [10,11]. Prior studies demonstrated that current injections into cardiac muscle...
cells using voltage clamp techniques can modulate contractility by modulating amplitude and/or duration of depolarization [12–15]. Such contractility modulation has been suggested to result from enhancement of trans-sarcolemmal calcium flux with calcium loading of the sarcoplasmic reticulum [13,14,16]. However, since voltage clamp techniques are not applicable to the intact heart, this approach had not previously been pursued in a clinically relevant manner.

It has recently been shown that similar to voltage clamping, extracellular electrical stimuli delivered during the absolute refractory period (ARP) can also induce inotropic effects both in-vitro and in-vivo (Cardiac Contractility Modulation; CCM) [17–19]. Since this modulation is achieved by stimulation during the ARP, no additional action potential is generated thus avoiding arrhythmogenicity and impairment of relaxation encountered during paired pacing [20,21]. This approach has been adapted for use in patients, and preliminary studies have provided the necessary feasibility and safety data necessary to launch larger scale studies of this approach [22–25]. However, the cellular mechanisms by which extracellular CCM signals modulate myocardial contractility are only partially investigated [19]. Prior studies describe the basic phenomenon that CCM signals can enhance myocardial contractility and discuss the possibility that this effect is mediated by an influence on the action potential which in turn acts to enhance peak systolic calcium [19]. In the present study we further explore the feasibility of this mechanism by testing how the amplitude and duration of the CCM signal influence cardiac contractility and testing the role of sarcosomal calcium channels, calcium loading of the sarcoplasmic reticulum and β-adrenergic stimulation as possible mechanisms underlying CCM signals effects. Finally, the effects of CCM signals on failing human myocardium are shown to be similar to those identified in normal rabbit myocardium.

2. Methods

The investigation was conducted in accordance to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (1996). Experiments were carried out on rabbit RV papillary muscles obtained from anesthetized (Nembutal, 50 mg/kg iv), anticoagulated (heparin, 2000 IU iv) New Zealand White rabbits (~2 kg) whose hearts were quickly removed and transferred into a dissection bath. The RV was opened and thin, long papillary muscles (diameter 0.8 mm, length 3.3±0.3 mm) were excised.

Muscle strips were placed in a muscle bath, held by a plastic holder at the proximal end and tied at the tendinous end by a silk thread to a rigid hook connected to the force transducer (isometric force transducer model F30 Type 372 coupled to DC-Bridge Amplifier model 660, HSE, March-Hugstetten, Germany) (Fig. 1). Muscles were superfused (10 ml/min) with modified Krebs-Henseleit solution (CaCl₂ 2.5 mM, pH 7.40, 37 °C). Muscles were equilibrated at a pacing rate of 1 Hz (2 ms, 1 mA pulses) at slack length for 30 min and then gradually stretched to provide maximal tension development (Lmax). Identical methods were used to isolate, prepare and study endocardial trabeculae obtained from three human hearts explanted at the time of orthotopic heart transplantation.

Cardiac contractility modulation signals (CCM, described below) were generated by a computer controlled constant current source and were applied between platinum–iridium electrode (diameter 0.125 mm; cylindrically coated by Teflon positioned ~0.5 mm from the muscle) and two graphite electrodes placed at the far ends of the organ bath (Fig. 1, CCM lead and G1 and G2 reference leads, respectively). Transmembrane potentials were recorded using glass micropipette (filled with 3 M KCl, tip resistance 15–25 MΩ) using an AxoClamp 2B amplifier (Axon Instruments, Foster City, CA, USA). Action potential duration at 80% repolarization (APD80) was determined from these recordings.

2.1. CCM signals

CCM signals consisted of monophasic square wave current pulses with peak amplitudes ranging between 3 and 8 mA (1.5–4.5 times the pacing threshold), with total duration ranging between 20 and 40 ms. Signals were timed to be delivered 20–40 ms after the pacing signal. (Except when specified otherwise.)

2.2. Data acquisition and analysis

Data sampling was carried out using National Instruments DAQ acquisition board (DAQCard AI-16E-4, National Instruments, Austin, USA) at a 1-kHz sampling rate. The acquired data were saved on computer disk for offline analysis using custom programmed software (MatLab). Values obtained prior to and during CCM application were compared using t-tests of means; \( p < 0.05 \) was considered significant.
3. Results

3.1. CCM signal effect on contraction and action potential duration

Negative amplitude (cathodic) CCM signals induced early repolarization and negative inotropic effects whereas positive amplitude (anodic) CCM signals prolonged depolarization and enhanced contractility (Fig. 2A and B). The changes in action potential duration (APD) started on the first beat of CCM delivery, were maintained throughout the duration of CCM signal delivery and returned to baseline on the first beat following CCM removal (Fig. 2C and D, lower tracings).

Contractility was affected on the first beat of CCM application and the magnitude of the effect increased gradually (Fig. 2C and D, middle tracings), reaching a steady state within 4–8 beats. Similarly, contractility returned to baseline within 6–10 beats after CCM signal termination.

The effect of CCM parameters on contractility and APD were evaluated at various signal amplitudes, durations and delays from the pacing stimulus (Fig. 3). Over the range of parameters tested, steady-state contractility and APD80 showed positive correlations with each of these parameters.

The effect of CCM parameters on APD80 and contractility exhibited similar patterns.

The characteristics of the CCM inotropic effects are further summarized in Table 1. Anodic CCM signals (delay 30 ms, duration 30 ms and amplitude 4.3 mA) substantially increased peak developed force and slightly prolonged time-to-peak force, lengthened twitch duration, and increased the rate of relaxation and the relaxation time (RT) measured from the point of peak force to 50% decline in developed force (RT50%). In contrast, cathodic pulses (delay 30 ms, duration 30 ms and amplitude 2 mA) decreased peak developed force and slightly decreased time-to-peak force, decreased twitch duration and decreased the rate of relaxation rate and RT50%.

Although both positive and negative effects on contractile force can be achieved with CCM signals by appropriate adjustment of parameter values, the remainder of the work to be described will focus exclusively on the positive CCM signals, which enhance contractility.

3.2. Role of calcium channels

To assess the role of L-type calcium channels in mediating the contractile response, CCM signals were applied at

![Fig. 2. Effect of cathodic (A and C) and anodic (B and D) CCM stimuli on isometrically contracting rabbit papillary muscle. CCM signals cause instantaneous changes in action potential duration followed by gradual effects on contractility. Upon stimulus termination, effects subsides to baseline with similar kinetics. Panels C and D show CCM effects of panels A and B, respectively, on expanded time scales. Upper tracings in each of these panels show CCM stimuli delivery, where positive represent anodic stimuli and negative represent cathodic stimuli. Lower tracings show isometric force generation. Bottom traces show membrane potential. Blue represents baseline traces and red traces are measurements made during CCM signal delivery.](image-url)
baseline and following exposure of the muscle strips to verapamil (20 μM, n = 7). Under baseline conditions, CCM impulses increased developed force by 53 ± 5% (from a mean value of 227 to 345 mg/mm²). Upon exposure to verapamil, baseline force decreased significantly (to 43 mg/mm²) and the same CCM signals increased force by only 40 ± 6% (to 58 mg/mm²). Thus, L-type calcium channel blockade blunts CCM signal effects.

3.3. Role of the sarcoplasmic reticulum (SR)

To assess the role of the SR in the inotropic effect of CCM signals, ryanodine (1 μM; n = 7) was applied, thereby inducing an open block in a sub-maximal conductance state of the ryanodine channel and inhibiting the participation of the SR in the excitation–contraction coupling. Prior to ryanodine administration (Fig. 4A, gray tracing), the typical inotropic effects of CCM were observed with isometric peak force increasing gradually by 56.0 ± 8.7% of the baseline value and relaxation rate improving, as indexed by a 3.2 ± 1.4% shortening of RT50% (p = 0.031) and a 69.1 ± 9.0% (p = 0.007) improvement in peak negative dT/dt. As described above, an initial inotropic effect is seen on the first beat of CCM application, the effect gradually rises until a steady-state contractility is achieved within 4–8 beats, which starts to decay on the beat following withdrawal of the signal.

Exposing the muscle to ryanodine (1 μM, Fig. 4A, red tracing) decreased baseline steady-state contractile force by 48.2 ± 9.5% (p = 0.004), and the increase in force in response to CCM signals diminished to only 10.3 ± 4.2% Additionally, effects on relaxation decreased (0.8 ± 1.0% reduction in RT50% and only 14.2 ± 2.2% increase in peak negative dT/dt). Under these conditions, the positive inotropic effect of CCM signals was present on the very first beat of signal application with no further rise on subsequent beats. Although the maximal magnitude of the CCM inotropic effect was reduced, the percent increase of contractility on the first beat of CCM delivery was similar with or without ryanodine. The CCM signal increased contractility by 11.4 ± 1.9% on the first beat under control conditions and by 11.2 ± 3.6% following exposure to ryanodine, and contractility returned to baseline on the first beat after withdrawal of the CCM signal in the presence of ryanodine.
To further assess the role of the SR, changes in force were measured when CCM signals were applied on every other beat (Fig. 4B). This produced a sequence of alternately strong and weak contractions. Importantly, the weak beats were coincident with CCM signal application while the strong contractions occurred on beats not receiving the CCM signal. As detailed in Discussion, this finding suggests that CCM signals induce SR calcium loading on the beat on which CCM signals are applied.

### 3.4. Role of beta-adrenergic receptors

To investigate the possibility that CCM inotropic effects are mediated by stimulation of sympathetic nerve terminals...
to release norepinephrine (1,3), CCM signal effects were tested in the presence of high dose β-blockade (propranolol, 0.5 μM; n = 4). Basal isometric force production was depressed by 39.3 ± 7.3% of control by this concentration of propranolol. However, the percent increase in developed force was similar prior to and during exposure to propranolol, averaging 55.6 ± 9.4% and 57.4 ± 9.1%, respectively. Similarly, the effect of CCM signals on RT50% (3.3 ± 1.1% vs. 2.1 ± 2.1% shortening; p = NS) and the increase in peak negative dT/dt (67.3 ± 1.5% vs. 74.7 ± 7.1%, p = NS) were not significantly affected by β-blockade.

3.5. Failing human myocardium

SR function is impaired in heart failure raising the possibility, in view of results presented above, that the CCM signal may not effectively improve contractile force in that setting. Ventricular trabeculae obtained from human hearts explanted from six patients with severe heart failure at the time of transplantation were placed in a muscle bath and studied in a manner similar to that of the rabbit papillary muscles (cross sectional area 0.64 ± 0.23 mm²). As in the normal rabbit papillary muscles, inotropic effects were dependent upon CCM signal polarity and amplitude. As seen in Fig. 5, positive polarity signals increased contractility almost linearly with amplitude whereas negative polarity signals depressed contractility. Also as in normal rabbit myocardium, contractility was affected on the first beat of CCM application, rose gradually, reaching a steady state within 4–8 beats and returned to baseline also within 4–8 beats after CCM signal termination. For similar CCM parameters (duration 40 ms, delay 60 ms and amplitude 4.8 mA) there were slightly decreased inotropic responses in the human myocardium as compared to the normal rabbit myocardium (Table 1). Nevertheless, there were significant increases in peak isometric force induced by CCM signals. The improvement in contractility was not associated with significant changes in RT50% (which increased non-significantly by 1.3 ± 1.9%, p = 0.489) while negative dT/dt increased by 16.8 ± 4.1% (p = 0.032). Resting tone was unaffected by CCM signals.

4. Discussion

The present study demonstrates that application of extracellular electrical signals during the absolute refractory period can exert effects on action potential duration, contractility and relaxation of cardiac muscle via cellular mechanisms that involve SR calcium loading. The effects are shown to be present not only in normal rabbit muscle, but in cardiomyopathic human muscle as well. These results are in line with early studies using voltage clamp techniques which showed previously that current injections during the early plateau phase of the action potential can prolong depolarization and enhance contractility due to an increase
in net trans-sarcolemmal calcium entry that gradually loads the SR with calcium [12–15,26]. This mechanism is supported by the finding that calcium channel antagonism reduces the relative magnitude of the CCM effect on contractility. The findings are also in line with recent studies of failing canine myocardium showing that action potential duration can exert effects on the amplitude and duration of the calcium transient [27].

A correlation between changes in the duration of depolarization and contractile responses has been identified in several early studies [14,15,26,28]. Three possible mechanisms have been proposed: (1) increased calcium entry through sarcolemmal voltage dependent calcium channels (mainly L-type), (2) decreased calcium extrusion via the sodium–calcium exchanger and (3) enhanced calcium entry via sodium–calcium exchanger working in reverse. All three mechanisms could be operative at the same time and can also account for the reverse effects (i.e., negative inotropic effects due to intracellular calcium depletion) observed with action potential shortening during cathodic currents.

Current theories of cardiac muscle excitation–contraction coupling indicate that calcium enters the cell during the action potential plateau (mainly via L-type channels), which induces release of larger amounts of calcium from the SR (calcium-induced calcium release) [29]. Calcium entering through the sarcolemma predominantly enters the SR to become available for release on subsequent excitations [30] and only a small amount goes directly to the myofilaments to contribute to contractility on the same beat.

This concept was partly generated from an observation of the voltage clamp technique, when the duration of depolarization was prolonged on every other beat, strong beats alternated with weak beats, with strong contractions occurring on beats with shorter depolarization. This phenomenon indicated that the extra calcium entering during the prolonged depolarization is largely sequestered by the SR and released on the subsequent beat, resulting in a stronger contraction. In the present study, we made a similar observation, when CCM signals were delivered on every other beat, stronger contractions occurred on beats not receiving CCM (Fig. 4B).

The involvement of the SR was further demonstrated in our study by the exposure to ryanodine, which eliminated the gradual rise in contractility and blunted the steady-state inotropic effects of CCM signals. The inotropic effects of CCM signals observed on the very first beat of signal application are non-SR dependent and presumably induced by the portion of enhanced sarcolemmal calcium entry that goes directly to the myofilaments. Interestingly, similar to previous reports using voltage clamp techniques, inotropic effects on the very first beat of CCM delivery were insensitive to ryanodine [13]. In addition, during exposure to ryanodine, inotropic effects disappeared on the first beat of CCM withdrawal, whereas under normal conditions without ryanodine, the effects began to decay on the second beat of CCM withdrawal (Fig. 4). All the above observations provide evidence to support the hypothesis that, similar to the CCM effect of the voltage clamp technique, the SR accumulates extra calcium entering the cell during the CCM impulse to increase the sarcoplasmic release of calcium during the subsequent systole. However, whether CCM effects the sodium calcium exchange mechanisms has not been studied as of yet and could also be a factor; further study of this mechanism is warranted.

The cellular processes involved with calcium handling (and specifically the SR) are known to be abnormal in heart failure [11,31,32], raising the question as to whether CCM signals would be effective in that setting. To address this concern, we studied the effects in human trabeculae isolated from end-stage failing hearts obtained at the time of transplantation. Studies performed in diseased human myocardium show qualitatively and quantitatively similar effects on contractile performance. In particular, contractile force changes only a little on the first, then builds beat-by-beat to a new plateau within about 10 beats. The decay of contractile force upon removal of the CCM signal mirrors that observed during signal initiation. The effects varied with amplitude of the CCM pulse. These observations indicate not only that the CCM effect is operative in end-stage cardiomyopathy and also suggests (although does not prove) that the mechanisms of action in this setting are similar to those identified to be important in normal myocardium discussed above.

The fundamental mechanisms of the inotropic effects of CCM signals are substantially different from postextrasystolic potentiation or paired pacing. Unlike these other inotropic maneuvers, CCM stimuli can reduce the contractility, exhibit a dose dependent correlation with signal amplitude and, most importantly, does not involve initiation of an extra action potential as proven by direct measurement of transmembrane action potentials [14,30].

Efferent nerve endings are present throughout the myocardium. High electric currents (50 mA–100 mA) have been suggested to stimulate these nerve terminals causing regional norepinephrine release, which may be a mechanism of positive inotropic effects induced by a non-excitatory stimuli of the voltage clamp technique [33,34]. However, several observations rule out this possibility regarding the CCM stimuli. First, CCM effects on contractility and relaxation were preserved in the presence of propranolol (0.5 μM). Second, CCM inotropic effects showed a strong dependence on the time delay from excitation. Third, negative inotropic effects could be achieved when the polarity of the CCM stimuli was reversed. Finally, response to the CCM stimuli delivered on every other beat yielded an alternating inotropic response, with strong contractions observed on the beats not receiving the signal. Moreover, the literature describing electrical stimulation of myocardial adrenergic nerve terminals, in a comparable set-up, has reported that the related positive inotropic effect exhibits slower kinetics (minutes) and may be evaded if not adjusted for the purpose of nerve stimulation (i.e., train pattern) [34].
This is further supporting our distinction between adrenergic effect and CCM effect since CCM effect exhibits time scale of seconds.

Multiple clinical studies have shown that chronic inotropic therapy can be associated with increased mortality [6,8]. However, when patient condition deteriorates despite optimal medical therapy, short term in-hospital, inotropic agents are commonly used and are typically effective in improving fluid balance, renal function and symptoms. Thus, the development of safe therapeutic means of enhancing ventricular contractility, both pharmacologic and device based, is of great interest. This is particularly so in the modern era in which some have speculated that inotropic therapies may be safer in the presence of concomitant β-blocker therapy and implantable cardiac defibrillators (ICDs). In this regard, one may speculate that in-vivo CCM effects are local and may avoid systemic adverse effects, could be delivered intermittently and can be titrated to the needs of a patient by varying CCM signal parameters.

Preliminary reports of the ability of CCM signals to enhance contractility of the intact hearts of normal and failing canine models [17,18], as well as in patients with heart failure [22–25] have already appeared. Definitive studies of the safety and efficacy of this approach are underway.

In summary, the concept of applying extracellular electrical impulses during the refractory period to modulate contractility in intact and failing myocardium has been validated in this study of ex-vivo rabbit and human myocardium, respectively. The mechanism appears to involve direct modulation of the action potential duration with associated changes in trans-sarcolemmal calcium fluxes followed by modulation of the SR calcium content. Thus, the cellular mechanism of CCM appears to be analogous to a voltage clamp mediated phenomenon reported earlier, but in contrast to this, can be applied to the intact heart. These data indicate that further study of the possible therapeutic utility of CCM signals in the setting of heart failure is warranted.

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