



## Electric Currents Applied During the Refractory Period Can Modulate Cardiac Contractility *In Vitro* and *In Vivo*

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### Introduction

Current therapies for heart failure, including diuretics, digoxin [1], angiotensin converting enzyme inhibitors [2], spironolactone [3] and beta-blockers [4,5] have significantly improved patient survival and quality of life. However, both mortality and morbidity remain high in this condition and new therapies to address these problems are being sought. Since prior studies showed worsened survival with positive inotropic agents [6], this class of therapy is not being pursued extensively. On the other hand, despite improved survival, exercise tolerance is not significantly improved by beta-blockers [7]. On a mechanistic basis, it can be hypothesized that a safe, positive inotropic therapy (i.e., one that does not worsen mortality) that would augment cardiac output during times of exertion could improve exercise tolerance.

One example of a strategy to increase global ventricular contractile strength in patients with heart failure is so-called resynchronization therapy (reviewed in a companion paper). Poor coordination of the initiation of muscular contraction over the heart (due to long activation time and manifest as a widened QRS complex) is thought to decrease the strength of ventricular contraction beyond that resulting from the reduction of muscle strength [8]. By initiating the heart beat with a pacemaker connected to an appropriately positioned pacing lead or by simultaneous right and left ventricular activation, the synchrony of contraction can be improved resulting in an enhancement of global ventricular contractile strength [9,10]. Based upon the underlying mechanism, it is predicted that resyn-

chronization therapy would be most effective in patients with a long QRS duration at baseline, a prediction which has been substantiated in early studies of this therapy [11]. In addition, this therapy does not affect the contractility of individual muscles, leaving open the possibility that other therapies which do so could act synergistically in patients with long QRS durations or be effective as sole therapy in patients with normal QRS durations.

One of the primary cellular defects in heart failure of any cause is believed to be a reduction of peak intracellular calcium [12] due at least in part to down-regulation of genes encoding for the sarcoplasmic reticular ATPase-dependent calcium pump (SERCA2a) [13]. Early studies of isolated cardiac muscle showed that use of voltage clamping techniques to modulate the amplitude and duration of the action potential could enhance calcium entry and contractility in isolated papillary muscles [14]. However, investigation of this type of approach as a potential therapy for patients with heart failure has been undertaken only recently [15–18]. A conceptual breakthrough occurred with the recognition that voltage clamping techniques (which cannot be

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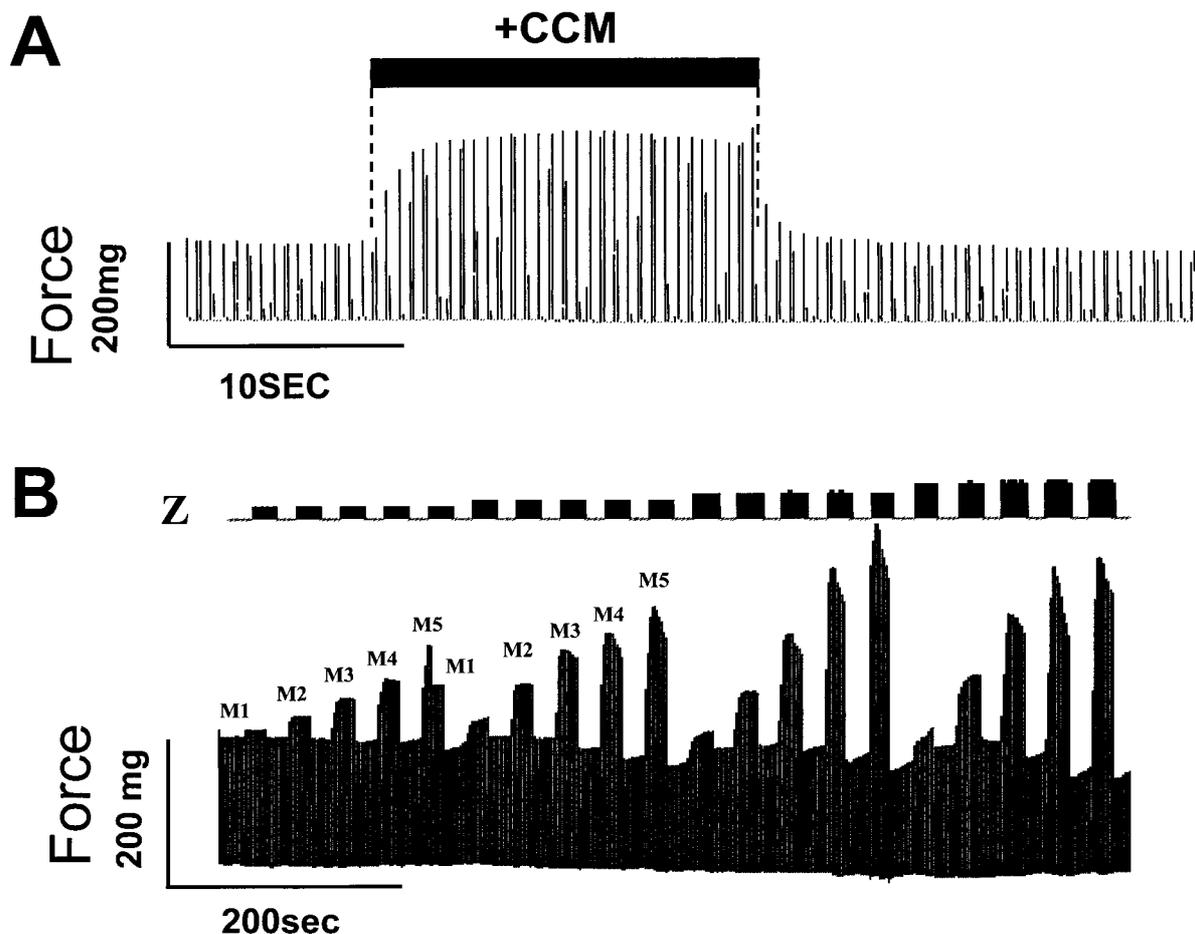
applied to the intact heart) were not required to influence calcium transients but rather, similar effects could be achieved with field stimulation of myocardial tissue. The basic data supporting the proposition that field stimulation can be used to enhance myocardial contractility in isolated muscle and in the intact heart are summarized in this report.

***Extracellular stimulations applied during the refractory period affect contractility and action potential duration***

To test the hypothesis that contractility could be modulated by extracellular electric fields, square wave current pulses were applied during the refractory period of contractions generated by a pacing stimulus in isolated, superfused, isometrically contracting rabbit papillary muscles. These signals are referred to as cardiac contractility modulation (CCM) signals because, as will be

shown, they influenced the strength of isometric contractions. Muscles were paced at 1 Hz by a 2 ms duration pulse of amplitude 3–5 times pacing threshold ( $\sim 1$  mA) by two electrodes placed in contact with the base of the muscle. The CCM signals were delivered between a single anode placed 0.5 mm away from the center of the muscle and two common cathode electrodes placed on the opposite side of the muscle  $\sim 1$  cm from each end to achieve extracellular stimulation of the muscle. As shown in Fig. 1A, force of contraction increased significantly within one beat following CCM signal application and reached a new steady state level after  $\sim 6$ –8 beats. This sequence was reversed after cessation of the signal. As shown in Fig. 1B, the amount of the inotropic effect can be affected by varying the amplitude (indicated by the parameter M) and by the duration (indicated by the parameter Z).

Along with the increase in contractile force, intracellular recordings have shown that the



**Fig. 1.** A. Representative isometric force tracings from a rabbit papillary muscle showing the rapid onset of inotropic effect due to the cardiac contractility modulating (CCM) signal. The decay of inotropic effect is similarly rapid. B. The magnitude of the inotropic effect is modulated by duration (Z) and amplitude (M) of the CCM signal.

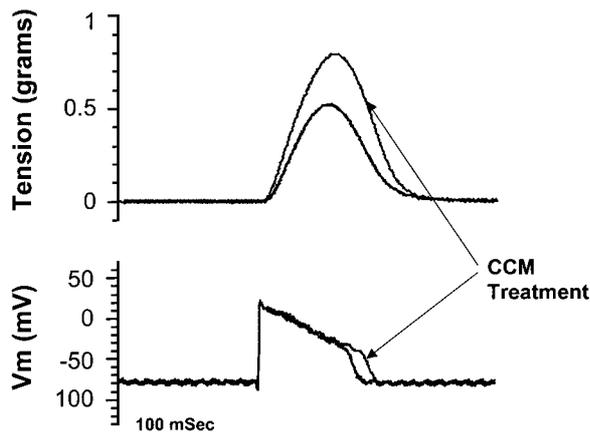
CCM signal increased the duration of the action potential as shown in Fig. 2 (note that the signal artifact due to the CCM has been removed in this tracing). It is hypothesized that this increase in action potential duration plus a potential effect of the CCM signal on the amplitude of the action potential are a mechanism by which calcium entry can be enhanced by CCM.

### CCM effects the calcium transient

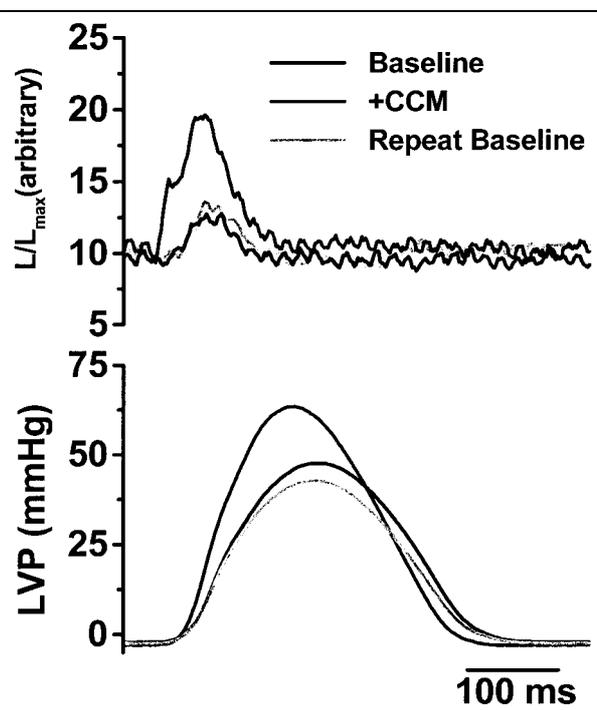
To test the hypothesis that changes in peak  $[Ca^{2+}]_i$  underscored changes in contractility, CCM signals were delivered to isovolumically contracting Langendorff perfused ferret hearts into which aequorin had been macroinjected in the inferoapical region.  $[Ca^{2+}]_i$  was estimated by normalized aequorin luminescence ( $L/L_{max}$ ). With hearts paced at 1 Hz and with extracellular calcium ( $[Ca^{2+}]_o$ ) set at 2 mM, the CCM signal significantly increased both peak  $[Ca^{2+}]_i$  and isovolumic pressure (Fig. 3). Thus, as hypothesized, the mechanism by which CCM signal enhanced contractility involved increasing calcium delivery to the myofilaments.

### CCM mechanism involves the sarcoplasmic reticulum

In cardiac muscle, calcium enters the cell during the plateau of the action potential through voltage sensitive L-type calcium channels. This initial rise in  $[Ca^{2+}]_i$  induces release of larger



**Fig. 2.** Isometric force (top) and transmembrane action potential (bottom) recordings under steady state baseline conditions and under steady state conditions during CCM treatment. The increase in contractile force generation is accompanied by an increase of action potential duration. Note a large electric potential due to the CCM signal itself is removed from the action potential recording; the presence of that signal obviates the ability to use standard micropipette recordings to determine the effect of CCM signal on the action potential amplitude during the time of signal application.



**Fig. 3.** Measurements in Langendorff perfused ferret hearts showed that application of CCM signals to the LV free wall increased isovolumic pressure generation (bottom) and peak intracellular calcium as indexed by light emission from macroinjected aequorin (top).

amounts of calcium from the sarcoplasmic reticulum (SR, calcium-induced calcium release) and itself enters the SR to become available for release on subsequent excitations. Calcium is removed from the cytosol by SR ATP-dependent pumps and by the sarcolemmal sodium:calcium exchanger.

To test the role of the SR in the inotropic effect of CCM signals, ryanodine, which inhibits the participation of the SR in excitation-contraction coupling, was applied to the papillary muscles. Prior to ryanodine application the usual inotropic effects of CCM were observed. Exposure of the muscle to ryanodine ( $1\mu\text{M}$ ) decreased steady state contractile force by about 50%. Following ryanodine exposure there was a relatively small increase in contractility on the first beat of CCM application and there was no further rise on the following beats. Under steady state conditions, contractility of isolated trabeculae can be increased by  $\sim 50\text{--}70\%$  by CCM signals, which contrasts to the less than  $\sim 20\%$  increase observed following ryanodine exposure.

Importantly, the blunted inotropic response following ryanodine is not simply a consequence of the decreased contractility. This was demonstrated in experiments in which basal isometric

force production was depressed by  $\sim 50\%$  by either a beta-blocker (propranolol) or a calcium channel blocker (nifedipine). Under these conditions of depressed contractility, the ability of the CCM signal to enhance contractility by 50–70% was retained. These data therefore suggest that the SR is involved in mediating the inotropic effects of CCM.

#### **CCM effect is additive to pharmacologic inotropic effects**

In addition to being able to enhance myocardial contractility when basal contractile strength is suppressed by standard negative inotropic pharmacologic agents, so too are the inotropic effects of CCM preserved when basal contractile strength is pharmacologically enhanced. Shown in Fig. 4 is the finding that the inotropic effect of CCM is additive to those of epinephrine at a concentration of  $0.5 \mu\text{M}$ . Shown in Fig. 5 is the finding that the inotropic effect of CCM appears to be synergistic with those of digitalis at a concentration of  $1 \mu\text{M}$ .

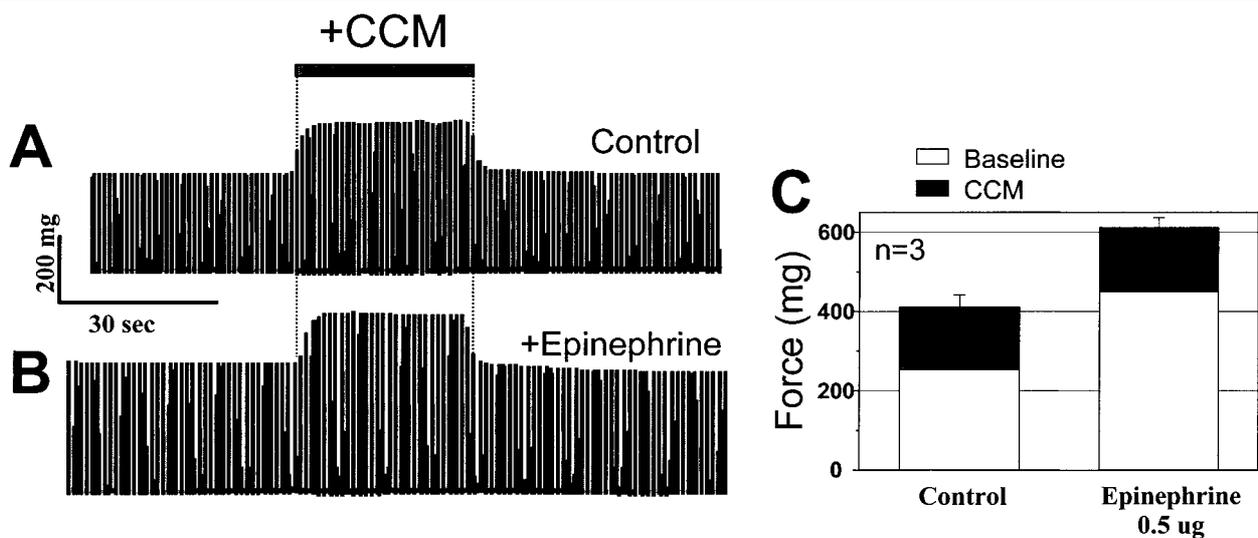
#### **CCM is effective in failing human myocardium**

Sarcoplasmic reticular function is known to be blunted in heart failure, raising the possibility that the CCM signal may not be as effective in improving contractile force in that setting. Trabeculae obtained from human hearts explanted from patients with severe heart failure at the time of cardiac transplantation were therefore

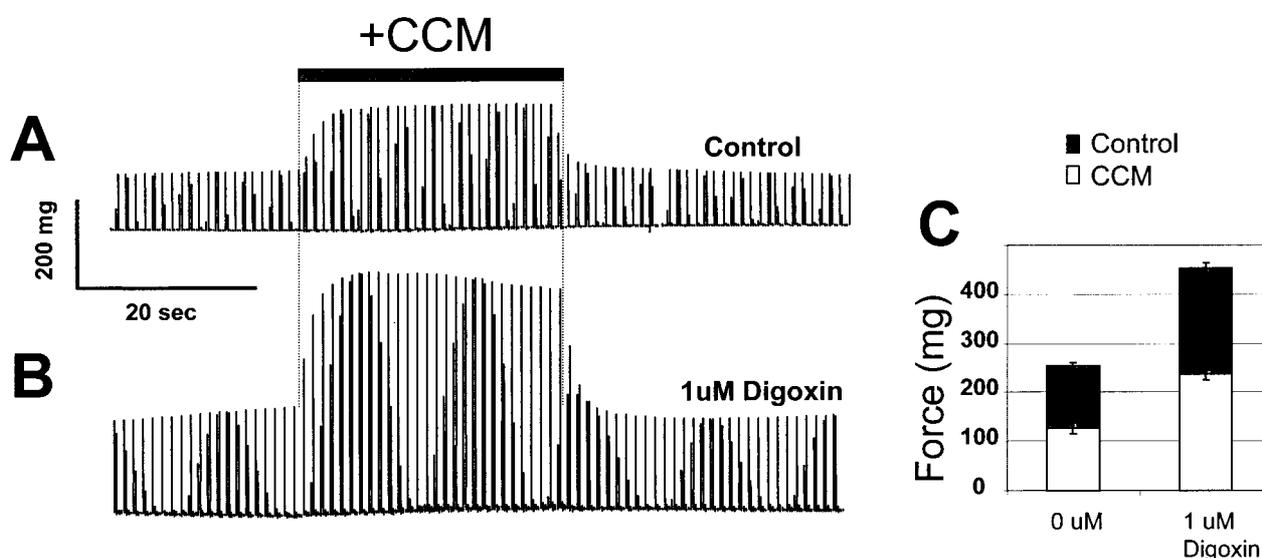
studied in a similar manner as were rabbit papillary muscles described above. As shown in the tracings of Fig. 6, application of CCM signals enhanced cardiac contractility in this preparation as well. The electrical signal artifact is superimposed on the force tracing to show clearly the time at which the signal is applied relative to the onset of contraction. On average, the CCM signal increased developed force by an average of approximately 30% from its baseline value in 6 human muscles studied in this manner.

#### **Regional application of CCM is effective in enhancing ventricular function *in vivo***

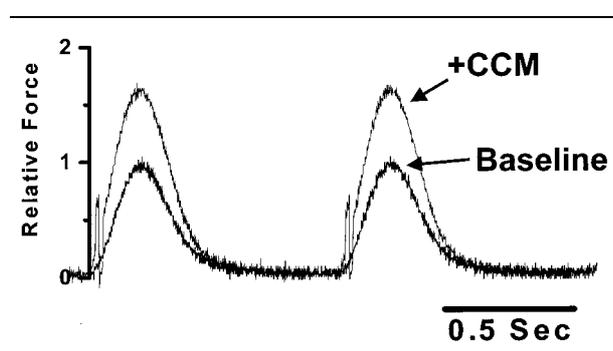
Extracellular electrical fields applied to papillary muscles, trabeculae and small Langendorff hearts are likely to reach and affect function of a large proportion of (if not all) muscle cells in the preparation. However, *in vivo* field stimulation of entire hearts of larger mammals is not feasible because of practical considerations related to power availability. Thus, we tested whether contractile strength of the heart could be significantly enhanced by CCM application to a region of the hearts of open chest anesthetized dogs. Two stitch electrodes separated by  $\sim 3 \text{ cm}$  were inserted into the myocardium of either the anterior or posterior wall of the heart. To ensure that the CCM signals were delivered during the local refractory period, a local bipolar electrogram was recorded by a separate set of electrodes so that the CCM signal could be delivered after detection



**Fig. 4.** Isometric force tracings from rabbit papillary muscles. A. Force tracings under baseline conditions showing inotropic effect of CCM signal. B. Force tracings from the same muscle after stimulation by epinephrine show similar amount of force enhancement. C. Summary of effect epinephrine and CCM on isometric force from three papillary muscles showing that the effects of these two inotropic interventions are additive.



**Fig. 5.** Isometric force tracings from rabbit papillary muscles. *A.* Force tracings under baseline conditions showing inotropic effect of CCM signal. *B.* Force tracings from the same muscle after stimulation by digoxin show slightly increased amount of force enhancement. *C.* Summary of effect digoxin and CCM on isometric force from three papillary muscles showing that the effects of these two inotropic interventions appear to be synergistic.



**Fig. 6.** Examples of isometric force generation of a human left ventricular trabecula from a patient with end-stage heart failure under baseline conditions and during stimulation with CCM signal.

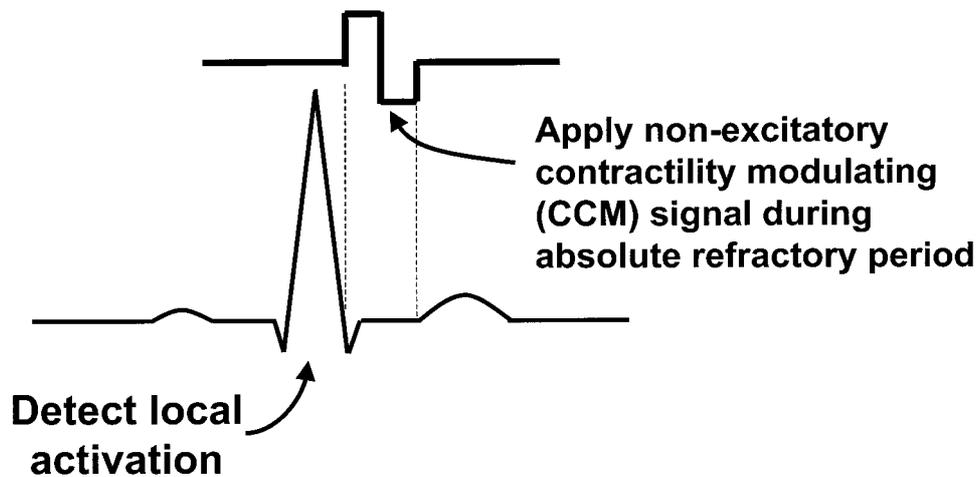
of local electrical activation (as illustrated in Fig. 7). To prevent polarization of the electrodes during long term use, a biphasic CCM signal of balanced charge was employed. The amplitude, duration and time delay from sensing local activity were adjustable. Left ventricular contractility was assessed by measuring end-systolic pressure-volume relationships (ESPVR) before and during CCM application (Fig. 8). In these studies, ventricular volume was measured by placing ~20 ultrasonic crystals (Sonometrics, London, Ontario) over the heart and estimating volumes from continuously tracked x-y-z coordinates of each crystal assuming an ellipsoidal ventricular geometry. A range of pressures and volumes was

achieved by performing a temporary vena caval occlusion. The ESPVR is determined by connecting the upper left corner of the pressure-volume loop of each beat during the series. The upward shift of the ESPVR during CCM application demonstrates that regional function can be sufficiently influenced to have a significant impact on global function.

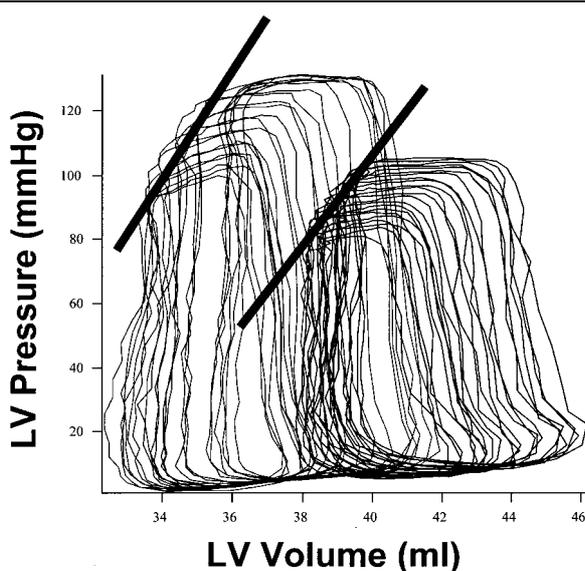
The data of Fig. 9 show the effect of repeated CCM signal applications on LV  $dP/dt_{max}$  and on peak aortic flow in an open chest dog. Signals were applied for ~20 minute periods with intervening ~10 minute off periods; this was repeated for approximately 2 hours. As shown, there was a repeatable significant effect on  $dP/dt_{max}$  and on peak aortic flow.  $dP/dt_{max}$  values during the intervening off periods increased slightly with each successive cycle, suggesting some accumulation of the inotropic effect of undetermined mechanism. With regard to peak aortic flow, there was a significant 'overshoot' on the very first CCM application which was not seen on subsequent applications.

## Discussion

It has previously been recognized that cardiac contractility can be modulated by actively controlling the duration and profile of the action potential which in turn influences calcium entry [14]. The results of recent studies indicate that application of extracellular electrical fields during the refractory period can exert similar



**Fig. 7.** Algorithm for applying CCM signals regionally to intact heart. A local bipolar electrogram is detected and the CCM signal is applied following a specified time delay after detection of the QRS complex.

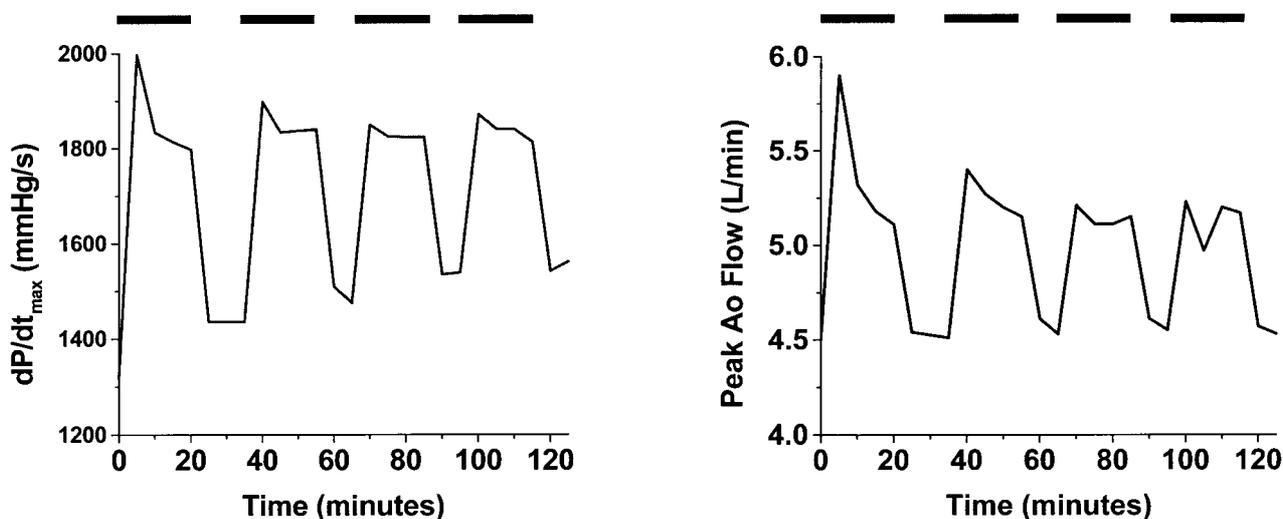


**Fig. 8.** Pressure-volume loops obtained during vena caval occlusions in open chest dogs under baseline conditions and during CCM signal application.

effects on action potential, calcium cycling and contractility in isolated papillary muscles. It has also been demonstrated that regional application of similarly timed currents to intact hearts can exert significant inotropic effects *in vivo*. These findings pave the way for development and testing of a new form of therapy for heart failure. Drug based therapies for heart failure have advanced significantly over the past 2 decades. Diuretics, digoxin and angiotensin converting enzyme inhibitors improve symptoms, enhance exercise tolerance and increase longevity. Spiro-

nolactone and beta-blockers have recently been shown to provide added survival benefits, though benefits of these agents on exercise tolerance is less clear [7]. While the full impact of these new therapies is not yet elucidated, it is recognized that patients continue to deteriorate clinically and they require repeated hospitalizations to treat heart failure exacerbations and exercise tolerance declines over time. Thus, current therapies are insufficient and new modalities for treating this large patient population are needed.

Until recently, the use of inotropic agents had been considered to be a logical approach to the treatment of heart failure. However, clinical studies have shown that chronic inotropic use can be associated with increased mortality [19]. However, when patient condition deteriorates (e.g., low cardiac output syndromes, fluid accumulation) despite optimal medical therapy, short term in-hospital use of inotropic agents remains the cornerstone of treatment in many institutions and is usually effective in improving fluid balance, renal function and exercise tolerance. Contractility enhancement by resynchronization therapy is currently being evaluated with promising preliminary reports [9,10]. Some reports are also beginning to emerge that suggest that pharmacologic based positive inotropic strategies can be safe and effective in patients with severe CHF (in combination with beta-blockers) [20], though larger scale trials are needed to substantiate these claims. Thus, it is recognized that development of safe therapeutic means of enhancing ventricular contractility, both pharmacologic and device based, could play a role in heart failure management and several approaches are being pursued.



**Fig. 9.** LV  $dP/dt_{max}$  and peak aortic flow values as a function of time with CCM signal repeatedly applied for ~20 minutes at a time over 2 hours. As shown, the inotropic effect is seen each time the signal is applied (bars).

The data presented in this review demonstrate that electric signals applied during the refractory period (so as to be non-arrhythmogenic) can influence cardiac contractility. Evidence is also presented demonstrating that the mechanism of contractility enhancement involves an increase in peak intracellular calcium. A role of the sarcoplasmic reticulum (SR) is also revealed by studies showing that inhibition of SR function by ryanodine administration blunts the inotropic effectiveness of CCM signals. The mechanism could therefore involve increased transsarcolemmal calcium fluxes or an influence of the electric currents on calcium release mechanisms from the SR. Since SR function is known to be depressed in heart failure, it was significant that failing human myocardium obtained from hearts explanted at the time of transplantation responded to these signals. It is also significant that application of CCM signals to a region of the heart *in situ* can influence regional contractility to such a degree that global function can be enhanced.

There are several important questions that must be addressed during the evaluation of this technology as a therapy for heart failure. Lack of pro-arrhythmic effects, ability to influence contractility in large dilated hearts, durability of the effect over long periods of time are among the important questions. With regard to the last point, it is envisioned that this form of therapy would be superimposed on drug therapies and could be incorporated into standard pacemakers or implantable cardioverter defibrillators. Since the therapy aims to enhance contractile strength

of the muscles, the inotropic effects and possibly the therapeutic effects of CCM signals could be additive to those of resynchronization therapy achieved by multisite ventricular pacing. Unlike resynchronization therapy, however, there is no requirement for conduction delay (prolonged QRS duration) so that if proven effective, this form of therapy could be applicable to a large number of patients.

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