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Effects of Hypocalcemia on Electrical Restitution and Ventricular Fibrillation

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Abstract—**We have shown previously that verapamil reduces the slope of the action potential duration (APD) restitution relation, suppresses APD alternans and converts ventricular fibrillation (VF) into a periodic rhythm. To determine whether these effects result primarily from reduction of the APD restitution slope, as opposed to alteration of calcium dynamics unrelated to restitution, we tested the effects of hypocalcemia ($[CaCl_2]$ = 31-125 μM) in canine ventricle. At normal $[CaCl_2]$ (2.0 mM), the slope of the APD restitution relation was ≥ 1, APD alternans occurred during rapid pacing and VF was inducible. During hypocalcemia the slope of the restitution relation remained ≥ 1 and the magnitude of APD alternans was unchanged. VF still was inducible and the mean cycle length and the variance of the FFT spectra during VF were not altered significantly. These results suggest that reduction of APD restitution slope, rather than blockade of I_{Ca} *per se*, is responsible for the antifibrillatory effects of verapamil in this model of pacing-induced VF, lending further support to the idea that APD restitution kinetics is a key determinant of VF.**

I. Introduction

It has been proposed that ventricular fibrillation (VF) is caused by the break-up of a single spiral wave into many self-perpetuating waves [1]-[3]. The mechanism for spiral wave break-up has not been established, but may involve destabilization of the wave secondary to rate-related alternans of action potential duration (APD) [4],[5]. The latter requires that the restitution relation for APD have a maximum slope of 1 or greater [6],[7]. Several experimental studies have provided evidence for a key role of APD restitution in the initiation and maintenance of VF [8]-[10]. In particular, we have shown that two drugs that reduce the slope of the APD restitution relation, verapamil and diacetylmonoxime, prevent the induction of VF and convert preexisting VF into a periodic rhythm [10].

Both verapamil and diacetylmonoxime block calcium current (I_{Ca}) [11],[12], and thereby alter intracellular calcium dynamics. Given that various aspects of calcium dynamics have been implicated in the development of VF [13]-[15], it could be argued that suppression of VF by calcium channel blockers is not caused by a reduction in the slope of the APD restitution relation, but by alteration of some aspect of intracellular calcium dynamics, independent of restitution. To distinguish between these two possibilities, we have in the present study taken advantage of the fact that hypocalcemia ($[CaCl_2]$ < 125 μM) markedly reduces I_{Ca} [11], but does not reduce the slope of the APD restitution relation [16]. Consequently, if suppression of VF by calcium channel blockers is caused by alteration of calcium dynamics, hypocalcemia should have effects similar to those of verapamil (i.e., it should prevent the induction of VF and convert preexisting VF into a periodic rhythm). Conversely, if the suppressant effects of verapamil are caused by a reduction in the slope of the APD restitution relation, then hypocalcemia should not suppress VF.

These possibilities were investigated by measuring the effects of hypocalcemia on: 1) the slope of the restitution relations for APD and conduction velocity (CV); 2) the induction of VF by rapid pacing and; 3) spatiotemporal organization during VF.

II. Methods

All experiments were approved by the Institutional Animal Care and Use Committee of the Center for Animal Resources and Education at Cornell University.

A. 2-dimensional preparations

Adult mongrel dogs of either sex, weighing 10-30 kg were anesthetized with Fatal-Plus (390 mg/ml pentobarbital sodium; Vortech Pharmaceuticals; 86 mg/kg i.v.) and their hearts were excised rapidly. Thin, rectangular (typically 5 mm x 20 mm) sections of right or left ventricular papillary muscle tips or trabeculae were superfused with Tyrode solution [9]. A bipolar stimulating electrode was placed at one end of the preparation and microelectrode recordings were obtained from 2 sites approximately 5 mm and 15 mm from the stimulating electrode.

Following equilibration in normal Tyrode solution, the $[CaCl_2]$ of the Tyrode solution was reduced from 2.0 mM to 125 μM (n = 6), 62.5 μM (n = 13) or 31.2 μM (n = 7). After a 30 minute equilibration period at the new $[CaCl_2]$, the relationships between APD and diastolic interval and between CV and diastolic interval were determined using a dynamic restitution protocol [9]. CV restitution also was measured in 6 preparations during exposure to normal Tyrode solution plus verapamil (2 μM). CV was measured as the distance between the 2 recording sites divided by the time between maximum upstroke velocities. CV restitution

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was expressed by plotting CV as a function of the diastolic interval at the recording site closest to the site of stimulation. APD restitution was expressed by plotting APD, measured to 90% of repolarization, as a function of diastolic interval at each recording site. The range of diastolic intervals over which the slope of the APD restitution relation was ≥ 1 and the magnitude of the APD alternans also were determined. Data obtained after exposure to different [$CaCl_2$] were compared using an ANOVA, followed by Scheffé's F-test, to determine statistical significance. Curve fitting was performed using TableCurve® (Jandel Scientific).

B. 3-dimensional preparations

Adult dogs were anesthetized as described above and their hearts were excised rapidly. The circumflex or left anterior descending coronary artery was cannulated and a transmural section of tissue was excised and suspended in a Plexiglas chamber, where it was perfused and superfused at a constant flow rate of 35 ml/min [10]. Epicardial electrical activity was mapped using an array of 64 monophasic action potential (MAP)-type recording electrodes and 1-2 microelectrodes.

The MAP signals were high-pass and low-pass filtered (cutoffs = 0.15 Hz and 250 Hz) and were sampled at 500 Hz with 12-bit resolution. To assess the degree of temporal organization during pacing and during VF, MAP data were analyzed using frequency spectral analysis, which included the generation of a composite FFT spectrum and the calculation of the average frequency and variance of the composite spectrum, as described previously [10].

In 14 preparations, the [$CaCl_2$] of the Tyrode solution was reduced from 2.0 mM to 125 µM (n = 5), 62.5 µM (n = 8) or 31.2 µM (n = 1) within 1-2 minutes after the onset of perfusion. After a 15 minute equilibration period at the new [$CaCl_2$], the pacing cycle length was shortened progressively until VF was induced. VF was monitored for an additional 15-30 minutes. In the 5 preparations exposed to [$CaCl_2$] = 125 µM, verapamil (2 µM) was added to the Tyrode solution after 15-30 minutes of VF had elapsed and VF was monitored for an additional 30 minutes. In 4 preparations exposed to [$CaCl_2$] = 62.5 µM, thapsigargin (1-2 µM) was added after 15-30 minutes of VF had elapsed and VF was monitored for an additional 30 minutes.

In 10 additional preparations, [$CaCl_2$] was maintained at 2.0 mM and VF was induced using rapid pacing. In 6 of the preparations, [$CaCl_2$] was changed to 125 µM (n = 3) or 62.5 µM (n = 3) after 15-30 minutes of VF had elapsed and VF was monitored for an additional 30 minutes. In the remaining 4 preparations, verapamil (2 µM) was added to the Tyrode solution after 5-15 minutes of VF had elapsed and VF was monitored for an additional 30 minutes.

III. Results

A. Effects of hypocalcemia on APD restitution

APD restitution was determined during superfusion with normal Tyrode solution and during hypocalcemia. Hypocalcemia ([$CaCl_2$] = 62.5 µM) prolonged APD at all pacing cycle lengths, as reflected by an upward shift in the dynamic APD restitution relation (Fig. 1) and by an increase in the mean APD at BCL = 400 msec, the shortest cycle length at which APD alternans did not occur in any of the cells (Table). Hypocalcemia had no significant effect on the maximum amplitude of APD alternans, but decreased the range of diastolic intervals over which APD alternans occurred (Table). Similar results were obtained for [$CaCl_2$] = 125 µM, and 31.2 µM (data not shown).

TABLE I
EFFECTS OF HYPOCALCEMIA ON APD ALTERNANS

[$CaCl_2$] (mM)	APD (msec)	AM (msec)	DI Range (msec)
2.0	191.7 ± 5.2	20.5 ± 2.0	64.3 ± 7.2
0.0625	237.3 ± 8.6*	26.1 ± 4.8 N.S.	40.1 ± 5.5*

Abbreviations: APD = action potential duration, AM = maximum magnitude of APD alternans, DI Range = range of diastolic intervals over which APD alternans occurred.
* P < 0.01 vs [$CaCl_2$] = 2.0 mM; N. S. = not significantly different; n = 13

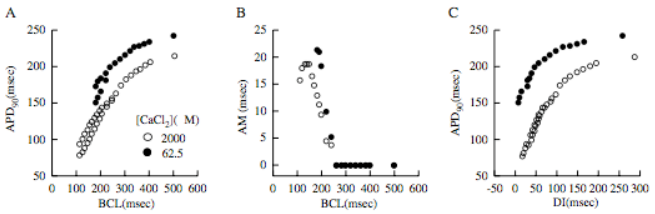

Fig. 1. Effects of hypocalcemia on APD restitution and APD alternans. *Panel A*. Example of the relationship between APD and pacing cycle length (BCL) for [$CaCl_2$] = 2 mM and 62.5 µM (see legend, lower right). *Panel B*. Relationship between the magnitude of APD alternans (AM) and BCL. *Panel C*. Relationship between APD and diastolic interval (DI).

B. Effects of hypocalcemia on CV restitution

CV restitution was determined during superfusion with normal Tyrode solution [$CaCl_2$] = 2.0 mM), alone or after the addition of verapamil (2 µM; n = 4), and after switching to hypocalcemic Tyrode ([$CaCl_2$] = 62.5 µM; n = 4). CV restitution relations in normal Tyrode before exposure to verapamil or to hypocalcemia were not significantly different from one another and were pooled for analysis. Neither verapamil nor hypocalcemia significantly altered the maximum CV at a pacing cycle length of 400 msec; CV (in m/sec) = 0.92 ± 0.06 for control, 0.83 ± 0.08 after verapamil and 0.99 ± 0.08 after hypocalcemia (mean ± SEM). In addition, the kinetics of CV restitution were not altered significantly by verapamil or by hypocalcemia (Fig. 2).

C. Effects of hypocalcemia on VF

Hypocalcemia did not prevent the induction of VF by rapid pacing in all preparations tested (n = 16). Once initiated, VF during hypocalcemia was characterized by aperiodic cellular electrical activity, as reflected by spatially and temporally disorganized monophasic action potential and transmembrane action potential recordings (Fig. 3). The mean frequency and variance of the composite FFT spectra during VF were no different for [$CaCl_2$] = 2.0 mM and

$[CaCl_2]$ = 62.5 µM (mean frequency = 12.9 ± 3.1 vs. 11.3 ± 2.3 Hz and variance = 0.0572 ± 0.0026 vs. 0.0566 ± 0.0041, respectively).

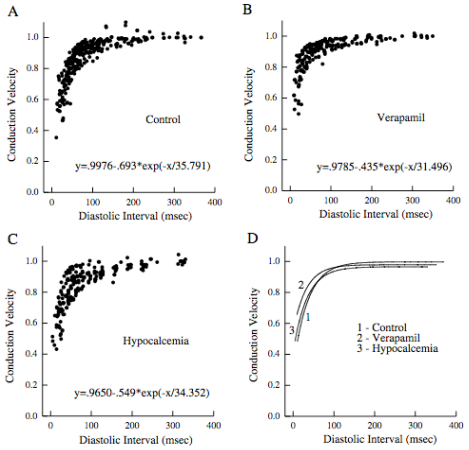

Fig. 2. Effects of hypocalcemia and verapamil on CV restitution. *Panel A*. CV restitution relations (with CV normalized to CV at BCL = 500 msec) for $[CaCl_2]$ = 2 mM (n = 8). The best-fit equation for the CV restitution relation is shown below the data. *Panel B*. CV restitution after 2 µM verapamil (n = 4). *Panel C*. CV restitution at $[CaCl_2]$ = 62.5 µM (n = 4). *Panel D*. Best-fit CV restitution relations for control (1), verapamil (2) and hypocalcemia (3).

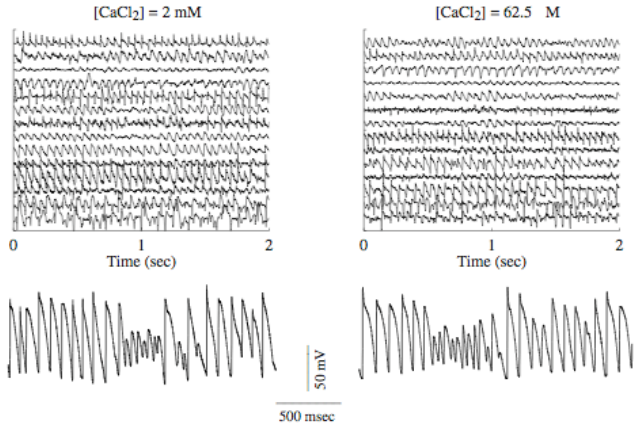

Fig. 3. Examples of MAP recordings (*upper panels*) and microelectrode recordings (*lower panels*) obtained 10 minutes after the onset of VF in isolated perfused canine left ventricle for $[CaCl_2]$ = 2 mM (*left panels*) and 62.5 µM (*right panels*).

Addition of verapamil converted VF to a periodic rhythm for $[CaCl_2]$ = 2.0 mM (n = 4), but not for $[CaCl_2]$ = 125 µM (n = 5) (Fig. 4). Addition of thapsigargin to hypocalcemic ($[CaCl_2]$ = 62.5 µM) Tyrode solution during VF (n = 4) had no apparent effect on VF, nor did reducing $[CaCl_2]$ from 2 mM to 62.5 µM (n = 3) or 125 µM (n = 3) (data not shown).

IV. DISCUSSION

A. New findings

In these experiments hypocalcemia, alone or in combination with drugs that decrease I_{Ca} (verapamil) or Ca^{2+} release from sarcoplasmic reticulum (thapsigargin), did not prevent the induction of VF or significantly alter spatiotemporal organization during VF. Hypocalcemia also did not reduce the slope of the APD or CV restitution relations or suppress APD alternans. The lack of an effect of hypocalcemia on APD restitution and VF contrasts with the effects of verapamil to reduce the slope of the APD restitution relation, suppress APD alternans, prevent the induction of VF and convert existing VF into a periodic rhythm. Thus, the antifibrillatory effects of verapamil appear to be mediated primarily by an effect on the APD restitution relation, independent of alterations in calcium dynamics. These results lend further support to the idea that the slope of the APD restitution relation is an important determinant of vulnerability to VF.

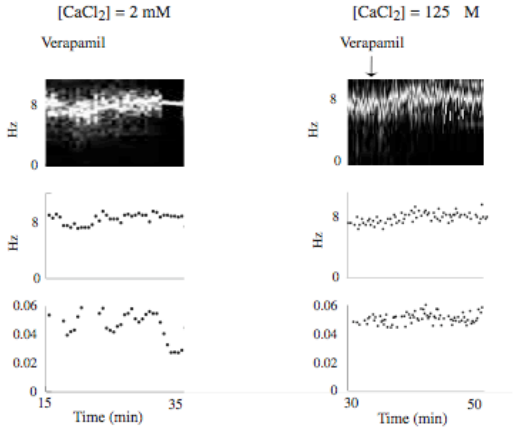

Fig. 4. Effects of verapamil (2 µM) on composite FFT spectra (*upper panels*), mean activation frequency (*middle panels*) and variance of the FFT spectra (*lower panels*) during VF for $[CaCl_2]$ = 2 mM (*left panels*) and $[CaCl_2]$ = 125 µM (*right panels*). Verapamil added at the arrows. Data shown beginning 15 (left) and 30 (right) minutes after VF onset. In $[CaCl_2]$ = 2 mM verapamil converted VF to a periodic rhythm (indicated by a single frequency band and a reduced variance at t = 30), whereas in $[CaCl_2]$ = 125 µM VF persisted.

B. Role of calcium dynamics in VF

Our observation that VF persisted during hypocalcemia, alone or in combination with verapamil or thapsigargin, suggests that transsarcolemmal calcium influx and release of calcium from the sarcoplasmic reticulum are not necessarily required for the initiation or maintenance of VF, provided APD alternans is maintained. Merillat *et al* [14] also found that hypocalcemia did not prevent the electrical induction of VF and that VF was not suppressed by ryanodine. In contrast to our results, however, they found that hypocalcemia suppressed existing VF. Moreover, hypocalcemia inhibited the induction of VF by sodium pump inhibition. Thus, the contribution of calcium dynamics to the development of VF may vary according to the experimental conditions [13]-[15],[17], as discussed in more detail elsewhere [18],[19].

The mechanism by which blockade of I_{Ca} suppresses VF also may differ according to the experimental circumstances. Samie *et al* attributed verapamil-induced conversion of VF to ventricular tachycardia in isolated rabbit hearts to a reduction of the dominant frequency during VF [20]. They postulated that the generator for VF is a single rotor. At high rotor frequencies, wavefronts emanating from the rotor are likely to encounter refractory tissue and fragment, producing fibrillatory conduction. Conversely, at lower frequencies rotor-generated wavefronts

are less likely to impinge on refractory tissue. Consequently, activation of the heart may be uniform, manifest as a monomorphic ventricular tachycardia.

In canine ventricle, however, verapamil initially increased the dominant frequency during VF, in association with an increase in spatiotemporal organization [10]. More prolonged exposure to verapamil resulted in the conversion of VF to ventricular tachycardia, at which time the dominant frequency was no different than before exposure to verapamil. Chorro *et al* also found that verapamil increased both the dominant frequency and the degree of electrical organization during VF in rabbit hearts [21]. These observations are consistent with the hypothesis that a reduction in the slope of the action potential duration restitution relation underlies the antifibrillatory effects of calcium channel blockers.

C. Role of calcium dynamics in APD alternans

The lack of effect of hypocalcemia on the magnitude of APD alternans was somewhat surprising, given the reported link between APD alternans and intracellular calcium dynamics following an abrupt change in pacing cycle length or the introduction of a premature stimulus [22],[23]. There also is evidence from modeling studies that alternation of I_{Ca} is a key determinant of electrical alternans during pacing at a fixed cycle length [15],[24]. However, APD was prolonged by hypocalcemia, despite a reduction of I_{Ca}, indicating that currents other than I_{Ca} must have been altered as well. This observation suggests that the ionic mechanism for APD alternans during hypocalcemia differs from that under normal conditions. The identity of that mechanism is unclear, however, as is the mechanism for prolongation of APD, although the latter may involve reduction of I_K, secondary to a shift in the voltage-dependence of activation to less negative membrane potentials [25].

D. Limitations

Because intracellular Ca^{2+} transients were not measured our preparations, we cannot be certain that hypocalcemia abolished transsarcolemmal calcium influx or calcium release from the sarcoplasmic reticulum. Consequently, the possibility that VF was sustained by a small residual calcium influx via I_{Ca}, calcium influx via reverse Na/Ca exchange or calcium release from intracellular stores cannot be excluded. Nevertheless, it seems reasonable to assume that transsarcolemmal calcium influx and release from the sarcoplasmic reticulum were inhibited by hypocalcemia to a similar or greater extent than by verapamil, yet verapamil in the presence of normal [$CaCl_2$] prevented the induction of VF and converted preexisting VF into a periodic rhythm, whereas hypocalcemia did not. These results strongly suggest that the antifibrillatory effects of verapamil cannot be attributed to suppression of calcium dynamics, independent of an effect on restitution.

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